MORPHOLOGICAL AND PHENOTYPIC CHANGES OF NOCICEPTIVE PRIMARY AFFERENT NEURONS FOLLOWING NEUROTMESIS AND PERINEURAL CAPSAICIN TREATMENT

Ph.D. Thesis

CSABA SZIGETI

Szeged

2011

MORPHOLOGICAL AND PHENOTYPIC CHANGES OF NOCICEPTIVE PRIMARY AFFERENT NEURONS FOLLOWING NEUROTMESIS AND PERINEURAL CAPSAICIN TREATMENT

Thesis for the Degree of Doctor of Philosophy

by

Csaba Szigeti

Department of Physiology, Faculty of Medicine
University of Szeged

Department of Cell Biology and Molecular Medicine
Faculty of Science and Informatics
University of Szeged

Szeged
2011

This thesis was based on the following publications:

I. JANCSÓ G, SÁNTHA P, **SZIGETI C**, DUX M, SELECTIVE C-FIBER DEAFFERENTATION OF THE SPINAL DORSAL HORN PREVENTS LESION-INDUCED TRANSGANGLIONIC TRANSPORT OF CHOLERAGENOID TO THE SUBSTANTIA GELATINOSA IN THE RAT. ***Neurosci Lett 361:204-207, 2004***

II. **SZIGETI C**, SÁNTHA P, KÖRTVÉLY E, NYÁRI T, HORVÁTH JV, DEÁK É, DUX M, GULYA K, JANCSÓ G, DISPARATE CHANGES IN THE EXPRESSION OF TRPV1 MRNA AND PROTEIN IN DORSAL ROOT GANGLION NEURONS FOLLOWING LOCAL CAPSAICIN TREATMENT OF THE SCIATIC NERVE IN THE RAT. ***Neuroscience*** 2011 Nov 9. [Epub ahead of print]

POSTERS:

I. **SZIGETI C**, KÖRTVÉLY E, SÁNTHA P, NYÁRI T, GULYA K, JANCSÓ G, CHANGES IN TRPV1 RECEPTOR EXPRESSION FOLLOWING PERINEURAL TREATMENT WITH CAPSAICIN AND RESINIFERATOXIN: IMPLICATIONS FOR THE ANALGESIC EFFECTS OF VANILLOIDS. ***5th Forum of European Neuroscience, Vienna, Austria, 2006 A215.224, 2006***

II. **SZIGETI C**, KÖRTVÉLY E, SÁNTHA P, NYÁRI T, GULYA K JANCSÓ G, EFFECTS OF TRAUMATIC AND SELECTIVE CHEMICAL LESIONS OF PERIPHERAL NERVES ON TRPV1 RECEPTOR EXPRESSION: IMPLICATIONS FOR VANILLOID-INDUCED ANALGESIA ***European Journal of Pain, 11, (1), 176, 2007***

Table of contents:

SUMMARY 2

1. INTRODUCTION 4

1.1. Morphological changes associated with peripheral nerve injuries 4

1.1.1. Effects of peripheral nerve transection 4

1.1.2. Effects of perineural capsaicin treatment 6

1.2. Chemosensitive primary sensory neurons 9

1.3. The function and regulation of the capsaicin receptor 11

2. THE AIMS OF THE STUDY 15

3. MATERIALS AND METHODS 16

3.1 Experimental animals 16

3.2. Peripheral nerve transection 16

3.3. Perineural capsaicin treatment 16

3.4. Intraneural injection of CTB- HRP 17

3.5. In situ hybridization 17

3.6. Quantitative RT-PCR measurements 19

3.7. TRPV1 immunohistochemistry 19

3.8. Semiquantitative densitometry 20

3.9. Classification of DRG neurons 20

3.10. Western blot analysis 21

3.11. Statistical analysis 22

4. RESULTS 23

4.1. Effect of neonatal capsaicin treatment on the lesion-induced alterations in the spinal distribution of myelinated primary afferent fibres 23

4.2. Localization of TRPV1 mRNA and protein in the L5 DRG of the rat 26

4.3. Effects of perineural capsaicin treatment or transection of the sciatic nerve on the expression of the TRPV1 mRNA in the L5 DRG of the rat. 29

4.4. Effects of perineural capsaicin treatment or transection of the sciatic nerve on the expression of the TRPV1 protein in the L5 DRG of the rat 35

5. DISCUSSION 39

6. ACKNOWLEDGMENTS 46

7. REFERENCES 47

SUMMARY

Chemosensitive primary afferent neurons which express the capsaicin/TRPV1 receptor play a pivotal role in the transmission of nociceptive stimuli toward the central nervous system. They are also intimately involved in the mediation of inflammatory and neuropathic pain and the neurogenic inflammatory response. The present Thesis summarizes our experimental findings concerning the participation of these particular class of nociceptive neurons in the mechanisms of the changes in the structural organization, neurochemical phenotype and gene expression of primary sensory neurons brought about by non-specific physical (nerve transection) and specific chemical (perineural capsaicin) lesions of peripheral nerves.

The findings disclosed the lack of a lesion-induced CTB-labelling of the substantia gelatinosa after the elimination of C-fibre chemosensitive primary afferent neurons by neonatal capsaicin treatment. This corroborates and extends previous reports suggesting that the increased labelling may be attributed to an uptake and transport of CTB by injured C-fibre primary afferent neurons, rather than to a sprouting response of A-fibre afferents. Since CTB binds selectively to the GM1 ganglioside and changes in neural gangliosides may affect the NGF-regulated expression of specific proteins of nociceptive primary afferents, the expression of the archetypic nociceptive ion channel, the TRPV1/capsaicin receptor was also investigated.

Quantitative morphometric and statistical analyses of L5 dorsal root ganglion cells revealed distinct populations of small (type C) and small to medium (type B) neurons which showed very high and moderate levels of TRPV1 mRNA and protein, whereas larger (type A) neurons practically did not express this receptor. Further investigations demonstrated that peripheral nerve lesions produced changes in these neurons which were markedly different in nature and depended on the type of the lesion inflicted upon the peripheral nerve. After either transection or capsaicin treatment of the sciatic nerve, immunohistochemistry and Western blotting demonstrated a massive (up to 80%) decrease in the proportion of TRPV1-immunoreactive neurons and TRPV1 protein at all postoperative survival times. After sciatic nerve transection, *in situ* hybridization indicated marked decreases (up to 85%) in the proportion of neurons which expressed the TRPV1 mRNA. In contrast, although perineural treatment with capsaicin resulted in similar substantial decreases in the proportions of type B and C neurons of the L5 dorsal root ganglia 3 days postoperatively, a clear-cut tendency to recovery was observed thereafter. Hence, the proportions of both type B and C neurons expressing the TRPV1

mRNA reached up to 70% of the control levels at 30 days postoperatively. In accord with these findings, quantitative RT-PCR revealed a marked and significant recovery in TRPV1 mRNA after perineural capsaicin but not after nerve transection. These observations suggest the involvement of distinct cellular mechanisms in the regulation of the TRPV1 mRNA expression of damaged neurons, specifically triggered by the nature of the injury. These findings also imply that the antinociceptive and anti-inflammatory effects of perineurally applied capsaicin involve changes in neuronal TRPV1 mRNA expression and long-lasting alterations in (post-)translational regulation.

The present findings may have important implications as concerns the mechanism(s) of chemically induced selective analgesia. The results point to the possibility that interfering with the translation and/or post-translational processing of nociceptive ion channels, such as the TRPV1 receptor, by using specific siRNAs, for example, may offer a novel approach to the production of antinociception by employing molecular biological tools.

1. INTRODUCTION

1.1. Morphological changes associated with peripheral nerve injuries

1.1.1. Effects of peripheral nerve transection

Different classes of primary afferent fibres terminate in a strict somatotopic and topographic manner in the spinal cord. Thick myelinated A-fibre afferents carrying mechanoreceptive information terminate in the deeper layers of the spinal dorsal horn, whereas capsaicin-sensitive, unmyelinated C-fibre nociceptive afferents project to the most superficial laminae, the marginal zone and the substantia gelatinosa (Jancsó and Király, 1980, Brown, 1981, Willis and Coggeshall, 1991). Neurotmesis, the most severe nerve injury which results in a complete division of a nerve, produces a loss of sensory, motor and autonomic functions (Seddon, 1943). Peripheral nerve transection represents a form of neurotmesis which results in Wallerian degeneration associated also with (transganglionic) degenerative changes within the central terminations of the injured (axotomized) primary sensory neurons (for reviews see Csillik and Knyihár, 1978, Aldskogius et al., 1985, Jancsó, 1992). Partial denervation of the spinal dorsal horn may produce changes in the neuronal microenvironment which, in turn, may promote axonal sprouting. Peripheral axotomy results in the appearance of degeneration argyrophilia in the somatotopically related central spinal projection areas of injured dorsal root ganglion (DRG) neurons (Grant and Arvidsson, 1975, Grant and Ygge, 1981, Aldskogius et al., 1985). Degenerative changes of some primary afferent terminals may induce reactive restorative phenomena in others producing a re-arrangement of dorsal horn neuronal connectivity (cf. Nagy and Hunt, 1983, Réthelyi et al., 1986). Accordingly, an apparently massive sprouting response of Aβ myelinated spinal afferents has been detected also after peripheral nerve transection in the adult rat. Indeed, following peripheral nerve transection, intense transganglionic labelling of the substantia gelatinosa by intraneurally injected choleratoxin B subunit (CTB) or its conjugates has been demonstrated (Woolf et al., 1992, Lekan et al., 1996, Nakamura and Myers, 1999, Kohama et al., 2000). Since CTB was regarded as a specific marker of myelinated fibres, this phenomenon was interpreted as a vigorous sprouting response of injured Aβ-myelinated afferents. It has also been suggested that this contributes to the development of chronic pain states (Woolf et al., 1992, 1995,

Nakamura and Myers, 1999, White, 2000). Recently, the sprouting hypothesis has been challenged by showing an increase in the proportion of CTB-horse radish peroxidase (HRP)-labelled small DRG neurons after peripheral nerve transection (Tong et al., 1999, Jancsó et al., 2002) and the co-localization of "injury peptides" such as vasoactive intestinal peptide (VIP) and galanin (GAL), characteristic of injured C-fibre DRG neurons, with choleragenoid in small sensory ganglion neurons and in their central terminations (Bao et al., 2002, Shehab et al., 2003). Electron microscopic histochemical studies furnished direct evidence for the transport of CTB-HRP in unmyelinated dorsal root axons following peripheral nerve transection (Sántha and Jancsó, 2003). It has been shown that local (perineural) capsaicin pretreatment of the transected nerve proximal to the anticipated tracer injection site prevented CTB-HRP labelling within the marginal zone and the substantia gelatinosa, but not in the deeper layers of the spinal dorsal horn. Perineural application of capsaicin results in a highly selective blockade of intraaxonal transport processes in capsaicin-sensitive unmyelinated C-fibre sensory nerves (Jancsó et al., 1980b, Gamse et al., 1982, Miller et al., 1982) which comprise up to 95% of all unmyelinated afferent fibres in rat lumbar dorsal roots (Nagy et al., 1983). In accord with these findings, many CTB-HRP-labelled neurons of various sizes were observed in ganglia relating to transected vehicle-treated nerves, whereas the proportion of small neurons was significantly decreased in ganglia relating to transected capsaicin-pretreated nerves (Sántha and Jancsó, 2003). These findings suggested that a phenotypic switch of C-fibre primary afferents rather than A-fibre sprouting may underlie the labelling of the substantia gelatinosa with CTB-HRP after peripheral nerve lesions (Jancsó et al., 2002, Sántha and Jancsó, 2003).

In addition to the long-term albeit reportedly reversible fine structural changes observed in the substantia gelatinosa and referred to as transganglionic degenerative atrophy (Csillik and Knyihár-Csillik, 1986), axotomy produces profound changes in the expression levels of neuropeptides and their receptors. The up- or downregulation of certain peptides may result from a change in the metabolic profile of the affected neurons, which might switch their metabolism to the synthesis of peptides and proteins critical for the structural and functional restoration of the injured cells (Lieberman, 1971, Hall, 1982, Tetzlaff and Kreutzberg, 1985). The term messenger plasticity (Hökfelt et al., 1994) describes a series of alterations which lead to a virtually new phenotype of the affected neurons. Primary sensory neurons are also good examples of coexistence systems, expressing both classical and other transmitters such

as nitric oxide (NO) and peptides (Hökfelt, 1991). The downregulation of the two main excitatory neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) was clearly demonstrated after axotomy (Nielsch et al., 1987, Noguchi et al., 1990, Dumoulin et al., 1991). However, in the same small-sized neuron population, an increase could be observed for the proteins and mRNAs of VIP and GAL which are practically undetectable under normal conditions (Shehab and Atkinson, 1986, Hökfelt et al., 1987, Xu et al., 1990). In parallel, elevated levels of neuropeptide tyrosin (NPY) and cholecystokinin (CCK) mRNAs and proteins were detected mostly in large diameter sensory neurons (Wakisaka et al., 1991, Verge et al., 1993, Zhang et al., 1994). After axotomy, there is a marked increase in nitric oxide synthase (NOS) mRNA and protein in those small diameter sensory neurons that upregulate GAL, VIP and NPY (Verge et al., 1992, Zhang et al., 1993). The observed downregulation of SP and CGRP results in the attenuation of transmission at the first synapse in the dorsal horn. However, it has been shown, that C-fibre transmission is maintained after axotomy (Wall et al., 1981). A possible explanation of this phenomenon could be that upregulated VIP takes over the role of SP and CGRP (Wiesenfeld-Hallin et al., 1990). Furthermore, some neuropeptides such as VIP and NO might promote survival of injured neurons by stimulating blood flow and other regenerative mechanisms (Hökfelt et al., 1994). Although the exact mechanisms are not well understood, it was suggested that NGF is important for the regulation of peptide expression (Lindsay and Harmar, 1989) which also depends on the type of the target tissue (McMahon and Gibson, 1987). The transient receptor potential vanilloid type 1 (TRPV1) receptor localized mostly in small chemosensitive primary sensory neurons is crucially involved in the transmission of nociceptive stimuli and pain (Jancsó et al., 1977, Caterina and Julius, 2001, Julius and Basbaum, 2001). Although capsaicin-sensitive primary afferents have been implicated in the mechanisms of neuroplastic changes which occur in the spinal dorsal horn after peripheral nerve injuries (Wall et al., 1981), systematic quantitative studies to reveal changes in the expression of TRPV1 expression after peripheral nerve lesions have not been performed.

1.1.2. Effects of perineural capsaicin treatment

Previous studies have demonstrated that selective elimination of the nociceptive afferents either from the whole animal or from selected regions of the body by systemic (neonatal) or localized (perineural) administrations of capsaicin and related vanilloids produced profound

antinociceptive and anti-inflammatory effects (Jancsó et al., 1977, 1980b, Fitzgerald and Woolf, 1982, Gamse et al., 1982, Jancsó et al., 2011). The perineural application of vanilloid compounds that results in a highly selective regional thermal and chemical analgesia has attracted much interest because of the possible therapeutic relevance of this intervention. Local application of capsaicin or resiniferatoxin has been shown to induce selective regional analgesia through long-lasting increases in the thresholds of nociceptive responses elicited by chemical irritants and intense heat stimuli (Jancsó et al., 1980a, Gamse et al., 1982, Chung et al., 1985). It also reduces inflammatory thermal and mechanical hyperalgesia, ischemic reactive hyperemia (Kissin et al., 2002, Domoki et al., 2003, Pospisilova and Palecek, 2006, Holzer, 2008, Jancsó et al., 2008, Oszlács et al., 2009) and arthritis (Donaldson et al., 1995). Antidromic vasodilatation and neurogenic inflammation, the cardinal local vascular responses of chemosensitive afferent endings brought about through stimulation with chemical irritants or antidromic stimulation of sensory nerves, are completely abolished by perineural capsaicin treatment (Jancsó et al., 1980b, Oszlács et al., 2009). The effects of the local application of capsaicin onto peripheral nerves can be separated into three phases (Jancsó and Such, 1983, Jancsó, 1992). The selective activation of C and Aδ nerve fibres producing neurogenic vasodilatation and extravasation is followed by the blockade of the impulse conduction in these fibres and finally, a complete chemical and marked thermal analgesia and abolition of neurogenic inflammation ensue. After perineural capsaicin treatment the retraction of Schwann cell processes from many unmyelinated axons leaving them packed closely together has been demonstrated but clear-cut, immediate degeneration of sensory C-fibres, unlike after systemic capsaicin treatment, was not observed (Jancsó et al., 1987). However, after longer survival periods the number of unmyelinated axons in the capsaicin-treated nerve was significantly reduced indicating a permanent loss of afferent axons from the treated nerve (Jancsó and Lawson, 1990, Pini et al., 1990).

The profound changes in the neurochemical phenotype and the subsequent decrease in the sensitivity of chemosensitive primary sensory neurons toward the neurotoxic effects of capsaicin are also characteristic features of perineural capsaicin administration (Jancsó and Lawson, 1988, 1990). The reduction in capsaicin sensitivity could be explained, at least in part, by a decrease in the level of the capsaicin receptor protein. However, there is little if any experimental support for this suggestion. The inhibition of intra-axonal transport of sensory neuron specific markers, such as SP, fluoride-resistant acid phosphatase (FRAP), thiamine

monophosphatase (TMP), CGRP and isolectin B4 (IB4, from *Griffonia simplicifolia)* was evident, but the transport of molecules characteristic of motor and autonomic nerves, such as acethylcholinesterase and noradrenaline was unaffected (Gamse et al., 1981, 1982, Jancsó and Lawson, 1988, Oszlács et al., 2009). In addition a reliable general marker of nerve injury, the transcription factor ATF3 exhibited a highly selective upregulation only in small diameter sensory neurons (Jancsó et al., 2011). Electrophysiological studies have revealed a selective and long-lasting reduction of impulse conduction in unmyelinated, but not in myelinated sensory axons after perineural capsaicin (Jancsó and Such, 1983, Baranowski et al., 1986, Pini et al., 1990), which was associated with a reduction of polymodal nociceptor units in the rat (Welk et al., 1983, Pini et al., 1990). Morphological investigations have disclosed a substantial, but partial reduction in the number of unmyelinated sensory (Baranowski et al., 1986, Jancsó and Lawson, 1990), but not autonomic (Jancsó and Lawson, 1987) axons in capsaicin-treated peripheral nerves and in skin areas innervated by a capsaicin-treated peripheral nerve (Jancsó et al., 1980a, Dux et al., 1998). Recent findings, however, indicated that that an ultrapotent analogue of capsaicin, resiniferatoxin may act differently since application of this agent to peripheral nerves induced lasting analgesia apparently without noticeable fine structural alterations in the rat (Kissin et al., 2002, 2007).

Perineural application of capsaicin results in a selective blockade of intraneuronal transport processes in unmyelinated C-fibres (Jancsó et al., 1980b, Gamse et al., 1982, Sántha and Jancsó, 2003). This may affect, among others, the retrograde axonal transport of nerve growth factor (NGF), a crucial regulator of TRPV1 expression, thus causing downregulation of the receptor and consequent loss or decrease in the sensitivity toward capsaicin (Jancsó and Lawson, 1988, 1990, Winter et al., 1988, Aguayo and White, 1992).

Despite the increasing body of experimental findings summarized in the previous sections, changes in the expression of the TRPV1 receptor, which confers capsaicin sensitivity on chemosensitive primary afferent neurons (Winter et al., 1988, Caterina et al., 1997, Michael and Priestley, 1999), have not been investigated so far after perineural treatment with vanilloid compounds. Therefore, one specific aim of the studies presented in this thesis was to reveal possible changes in the expression of the TRPV1 receptor following perineural capsaicin treatment and, for comparison, peripheral nerve transection.

1.2. Chemosensitive primary sensory neurons

Chemosensitive primary sensory neurons represent a unique population of peptidergic and non-peptidergic spinal and cranial sensory ganglion neurons which are sensitive to capsaicin and convey nociceptive impulses evoked by irritant chemicals, acid and heat. These neurons are also involved in the generation of the neurogenic inflammatory response which consists of neurogenic sensory vasodilatation and plasma extravasation (Jancsó et al., 1977, Jancsó and Király, 1980, 1981, Jancsó et al., 2011). Importantly, chemosensitive primary sensory neurons express the TRPV1 non-selective cation channel which confers the sensitivity to capsaicin onto these neurons (Caterina et al., 1997, Caterina and Julius, 1999, 2001).

Capsaicin was introduced in the study of pain and neurogenic inflammation by Miklós (Nicolaus) Jancsó in the 1940s, who demonstrated that capsaicin applied to the human skin causes burning pain and vasodilatation, followed by a phenomenon termed desensitization, which rendered sensory nerve endings insensitive to the pain-producing effects of capsaicin and other chemical irritants and also to heat (Pórszász and Jancsó, 1959, Jancsó, 1960, 1968 Jancsó et al., 1967). The chemical structure of capsaicin was reported as *trans*-8-methyl-*N*-vanillyl-6-nonenamid, an acryl-amide derivative of homovanillic acid by Nelson (Nelson, 1919). Capsaicin and other naturally occurring substances (e.g. resiniferatoxin, capsiate, gingerol, eugenol, piperine, cannabidiol) sharing homovanillic acid as structural motif were collectively named vanilloids (Szallasi and Blumberg, 1990, 1999, Szallasi et al., 1994, Liu and Simon, 1996, Bisogno et al., 2001, Witte et al., 2002, Bandell et al., 2004, Calixto et al., 2005). N. Jancsó was the first to postulate, that capsaicin acts on a specific pain receptor. Later, Gábor Jancsó (Jancsó et al., 1977) recognized the selective neurotoxic action of capsaicin by showing that systemic administration of the drug to neonatal and adult rats resulted in a selective degeneration of a distinct population of primary sensory neurons involved in the transmission of painful stimuli and in the mediation of the neurogenic inflammatory response (Jancsó et al., 1977). The demonstration of capsaicin sensitivity has become a useful anatomical, neurochemical and functional marker of a subset of primary sensory neurons processing noxious stimuli.

The chemosensitive primary afferent neurons which are selectively sensitive to the stimulatory and neurotoxic effects of capsaicin (Jancsó, 1968, Jancsó et al., 1977, Jancsó and Király, 1980) and express the TRPV1 receptor (Caterina et al., 1997, Caterina and Julius,

2001) play a fundamental role in pain mechanisms. They account for around 50% of DRG neurons and 95% of the unmyelinated dorsal root fibres in the rat (Nagy and Hunt, 1983, Millan, 1999). C-fibre associated nociceptors have slowly conducting (0.5-2 m/sec) axons and small cell bodies (< 400 μm^2). In contrast, Aδ-fibre associated nociceptors have medium-sized cell bodies (410–900 μm^2) and thin myelinated axons with intermediate conduction velocities (12-30m/sec). One group of C fibres, known as polymodal nociceptors respond to all three pain–producing modalities (mechanical, chemical, thermal), while others respond only to subsets of these. Morphologically, C-fibres can be divided into two main categories (Wood and Docherty, 1997). The so called peptidergic population contains pro-inflammatory peptides, such as SP and CGRP, and is regulated by NGF (Aguayo and White, 1992). The other population is non-peptidergic and can be identified histologically by the presence of TMP, FRAP or IB4 and postnatally they require glial cell line-derived neurotrophic factor (GDNF, Breese et al., 2005, Albers et al., 2006). A further class of nociceptors is referred to as "silent" or "sleeping" nociceptors (Cervero, 1994, 1995, McMahon et al., 1995). These nociceptors comprise 10-20% of unmyelinated C fibres in the skin, joints and viscera which are normally irresponsive to acute noxious stimuli. Under certain conditions (inflammation and tissue injury) they will be sensitized and activated by chemical mediators (Schmidt et al., 1995, Dmitrieva and McMahon, 1996). Upon exposure of the skin to a noxious stimulus, myelinated Aδ fibres elicit a rapid, first phase of pain, which is 'sharp' in nature, whereas unmyelinated C fibres evoke a second wave of 'dull' pain (Millan, 1999).

By virtue of their dual functional character, these particular nociceptive neurons comprise a unique population of primary afferent neurons (Jancsó, 1960, 1968, Jancsó et al., 1967, 1987, Maggi and Meli, 1988, Holzer, 1991, Jancsó, 2009). The transmission of itch and burning pain sensation towards the CNS is regarded as their afferent function. Upon stimulation they also release neuropeptides (e.g. CGRP and SP) from their peripheral and central terminals (Hökfelt et al., 1975, Gamse et al., 1982, Holzer, 1988, 1998, Sann and Pierau, 1998). The resulting local vascular responses involve vasodilatation and plasma extravasation, a phenomenon defined as neurogenic inflammation (Jancsó, 1960, 1968, Jancsó et al., 1968, 1980a). Injection of capsaicin into the cisterna magna results in a rapid degeneration of trigeminal and spinal chemosensitive afferent fibres and an abolition of nociceptive responses to chemical irritants, but leaves the efferent function (neurogenic inflammation) of the corresponding peripheral nerve endings intact. These findings provided direct evidence for the

dual function of chemo-/capsaicin-sensitive primary afferent neurons (Jancsó, 1981, Jancsó et al., 1984, Nagy et al., 2004).

The in vivo effects of capsaicin on the sensory neurons critically depend on the routes of application of the neurotoxin (Jancsó et al., 2011). Neonatal systemic (subcutaneous) administration results in complete abolition of the neurogenic inflammatory response (Jancsó et al., 1977, Gamse et al., 1980, Jancsó, 2009). The morphological and functional consequences of the same treatment in adult animals are less pronounced; only a subpopulation of capsaicin-sensitive sensory neurons degenerate (Jancsó et al., 1985). Such animals reveal changes in only 17 % of the lumbar DRG neurons with moderate decrease in neurogenic plasma extravasation (Jancsó et al., 1977, 1985). These systemic administrations produce complete or partial degeneration of the whole system of capsaicin-sensitive primary sensory neurons. In contrast, selective degeneration of different domains of capsaicin sensitive neurons can be achieved by the topical application or by the intrathecal and intracisternal injections of the neurotoxin. Topical capsaicin results in burning pain and thermal hyperalgesia, followed by loss of chemogenic pain sensation (Jancsó, 1960, 1968, Toth-Kása et al., 1983, Reilly et al., 1997, Nolano et al., 1999). Direct subarachnoid injections of capsaicin induce desensitization of the central sensory projections leaving the soma and the peripheral terminations intact (Yaksh et al., 1979, Jancsó, 1981, Palermo et al., 1981).

1.3. The function and regulation of the capsaicin receptor

The capsaicin receptor belongs to the transient receptor potential (TRP) superfamily of ion channels discovered by Minke, (1977). He identified a mutant *trp* locus in Drosophila displaying transient ion currents and abnormal Ca^{2+} influx in response to light stimuli, predicting that the related protein was an inward rectifying calcium ion channel (Montell and Rubin, 1989, Minke and Parnas, 2006). Besides photo transduction, evidence suggests that these TRP channels are operational in the mechanisms of controlling temperature, mechanosensation, pain, taste and pheromone detection (Cortright et al., 2007, Levine and Alessandri-Haber, 2007). Based on their amino acid sequence similarities, the TRP-related proteins fall into seven subfamilies (Benham et al., 2003, Nilius and Voets, 2004, Pedersen et al., 2005, Levine and Alessandri-Haber, 2007, Nilius et al., 2007). Six members of three

distinct TRP subfamilies (TRPV1–4, TRPM8 and TRPA1) are expressed in sensory neurons, thus they can be regarded as “sensory TRP” channels.

Almost half of the primary sensory neurons in the DRG express TRPV1 (Szallasi and Blumberg, 1990, 1999, Szallasi et al., 1994, Mezey et al., 2000). In the skin, TRPV1-positive fibres can be found in the epidermis and the Meissner corpuscules (Pare et al., 2001). In the gut, TRPV1 positive fibres distribute through the three mucosal layers, such as epithelium, submucosa and muscularis mucosae. They can be observed in myenteric plexi, where they establish synaptic contact with neurons. TRPV1 expressing cells can be identified in the villi too (Ward et al., 2003).

The identification and cloning of the grounding member of the TRPV subfamily, called TRPV1 or the capsaicin receptor (Caterina et al., 1997, Clapham, 1997) was of pivotal significance in the development of molecular pain research. TRPV1, the molecular integrator of noxious stimuli is a 95 kDa, 838 amino acid polypeptide with 6 TM segments and a pore region named P-loop within the extracellular linker between the 5^{th} and 6^{th} TM segments (Fig 1). It responds to vanilloids, acidic and thermal stimuli above 43°C (Tominaga et al., 1998, Caterina and Julius, 2001, Immke and Gavva, 2006). Vanilloid-binding sites (Arg-114, Tyr-511, Ser-512, Tyr-550, and Glu-761) and residues involved in proton-mediated activation (Glu-648) and sensitization (Glu-600) are depicted in Fig. 1. Glu-600, Asp-646, and Glu-648 are involved in cation-induced TRPV1 activation and sensitization (Tominaga and Tominaga, 2005). The glycosilation site of the receptor (Asn-604) provides the association with other proteins, so they together form major molecular complexes, called transducisomes or signalplexes (Chuang et al., 2001, Vennekens et al., 2002), which are important in the regulation of the trafficking and cytoplasmic membrane expression of the receptor (Nagy et al., 2004, Tominaga and Tominaga, 2005). The activation of the vanilloid receptor is coupled with the phosphorlyation through Ca^{2+}/calmodulin-dependent kinase II (CaMkII, Jung et al., 2004), whereas the desensitization occurs via dephosphorylation by protein phosphatase 2B (calcineurin, Docherty et al., 1996, Wu et al., 2005). The dynamic interaction of these enzymes with TRPV1 is responsible for the state changes of the receptor. The cAMP-dependent protein kinase (PKA) plays a major role in producing inflammatory hyperalgesia phosphorylating TRPV1 through the Ser116. PKA reduces the heat threshold of the receptor from 43 to 41°C and increases the response mechanism to other exo- and endogenous activators (Petho et al., 2004, Lee et al., 2005, Jeske et al., 2009).

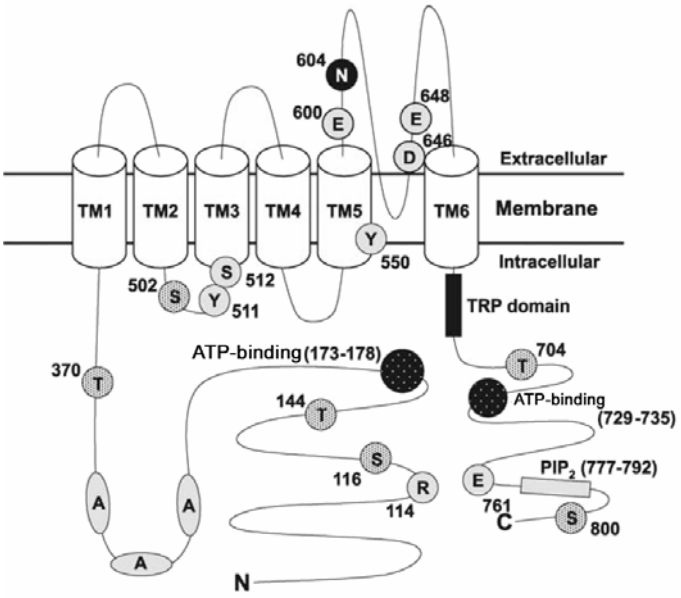


Fig. 1. Model depicting the membrane topology of TRPV1. The residue for *N*-glycosylation (Asn-604) is shown in black, phosphatidylinositol 4,5-bisphosphate (PIP2)-binding domain at the C-terminus is indicated as gray. Phosphorylation sites for PKA: Ser-116, Thr-144, Thr-370, Ser-502, for PKC: Ser-502, Ser-800 and for CaMkII: Ser-502, Thr-704 (Flockerzi, 2007).

PKA thus prevents TRPV1 desensitization (Bhave et al., 2002). Despite this important functional regulation, TRPV1 lacks any binding domain for PKA suggesting that PKA must act via scaffolding proteins in order to modulate the receptor activity. Phosphorylation through PKC activates the receptor and helps the sensitization to inflammatory mediators, such as bradykinin and ATP (Lee and Caterina, 2005, Lee et al., 2005). None of the known endogenous TRPV1 activators is able to induce channel opening in vivo on their own. It seems that they require pathological conditions to act in a synergistic manner. Protons, ATP, anandamide (the lipid mediator originally isolated from brain as an endogeneous cannabinoid ligand) are important components of tissue damage associated with infection or inflammation (Tognetto et al., 2001, Bianchi et al., 2006). The molecular mapping of the receptor residues revealed, that capsaicin binds to the residues in the field of the 3rd and 4th TM, the binding of resiniferatoxin requires an extra methionine. The same residues are the binding elements for the endogeneous vanilloids and for a competitive antagonist, capsazepine (Di Marzo et al., 2002, Starowicz et al., 2007).

The expression of the TRPV1 receptor has been demonstrated not only in the primary sensory neurons of the DRG, but also in various brain areas, such as the cortex, septum, hippocampus,

substantia nigra, cerebellum, several hypothalamic nuclei, central amygdala and the nucleus of the spinal trigeminal tract (Mezey et al., 2000, Toth et al., 2005). However, recent findings using genetically modified TRPV1 reporter mice have revealed, in contrast to reports of widespread and robust expression in the CNS, that neuronal TRPV1 is largely restricted to nociceptors in primary sensory ganglia, with minimal expression in a few discrete brain regions, most notably in a contiguous band of cells within and adjacent to the caudal hypothalamus (Cavanaugh et al., 2011).

Neurotrophic factor signaling has been thought to play an important role not only in the maintenance of the neuronal phenotype (Diamond et al., 1992), but also in the sensitization of sensory neurons under conditions of nerve injury(Lewin et al., 1993, Andreev et al., 1995, Pertens et al., 1999). NGF has been shown to regulate the sensitivity of a subpopulation of cultured DRG cells to capsaicin (Winter et al., 1988, Aguayo and White, 1992). Since many NGF-responsive neurons contain TRPV1, this channel is suspected of a role in NGF-mediated hypersensitivity (Caterina et al., 1997, Tominaga et al., 1998, Michael and Priestley, 1999). Cultured DRG neurons treated with NGF display enhanced inward current in response to capsaicin (Caterina et al., 2000, Zhu et al., 2004), whereas in the absence of the factor, they loose their capsaicin sensitivity (Winter et al., 1988, Winter et al., 1993). NGF can increase TRPV1 expression (Xue et al., 2007) and promote TRPV1 insertion into the plasma membrane (Zhang et al., 2005). The downregulation of TRPV1 mRNA has also been demonstrated after axotomy and it has been attributed to the decreased availability of local or target-derived NGF (Michael and Priestley, 1999)

2. THE AIMS OF THE STUDY

The experiments presented in this thesis were initiated in an attempt to further clarify the role of TRPV1 receptor expressing chemosensitive primary sensory neurons in central neuroplastic changes, induced by peripheral nerve injury and, in particular, to reveal the possible molecular mechanisms of capsaicin-induced chemical and thermal analgesia. The specific aims of the experiments presented in this thesis are as follows:

1. to furnish further evidence for and clarify the mechanisms of the contribution of capsaicin-sensitive primary afferents to the development of morphological changes which follow peripheral nerve injuries;
2. to classify the primary sensory neurons on the basis of the expression level of the TRPV1 receptor;
3. to provide direct evidence for changes in the expression of the capsaicin/TRPV1 receptor following non-specific traumatic and specific chemical lesions of peripheral nerves and,
4. to clarify the molecular mechanisms of the analgesic effects of capsaicin applied perineurally.

3. MATERIALS AND METHODS

3.1 Experimental animals

Adult male Wistar rats weighing 240–260 g at the start of the experiments were used in this study. The animal house was maintained under a 12-h light–dark cycle. All experimental procedures were approved by the Ethical Committee for Animal Care of the University of Szeged and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

3.2. Peripheral nerve transection

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). The right sciatic nerve was exposed high in the thigh and transected distal to a ligature. Sham-operated animals served as controls. After 3, 14 or 30 days, the animals were again anesthetized and sacrificed for immunohistochemical and *in situ* hybridization analyses.

3.3. Perineural capsaicin treatment

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). The sciatic nerves were exposed high in the thigh on both sides, and small pieces of gelfoam moistened with 0.1 ml of a 1% solution of capsaicin or the same volume of the vehicle were wrapped around the right and left nerves, respectively. After 20 min, the gelfoam pieces were removed, the wounds were closed and the rats were returned to the animal house. After 3, 14 or 30 days, the animals were again anesthetized and sacrificed for immunohistochemical and *in situ* hybridization analyses.

3.4. Intraneural injection of CTB- HRP

Animals were injected subcutaneously with a single dose of capsaicin (50 mg/kg, Fluka, Switzerland), or with its vehicle (8% ethanol, 6% Tween 80 in saline). Three months later, under chloral hydrate anaesthesia (400 mg/kg, i.p., Reanal, Hungary) the right sciatic nerve was exposed in the midthigh and transected distally to a ligature. Two weeks afterwards, the sciatic nerves were exposed and 1 ml of a 2% solution of CTB- HRP (Sigma) was injected into the nerves with a Hamilton microsyringe. Three days after the injection, the animals were deeply anaesthetized and perfused transcardially with an aldehyde fixative containing 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4), followed by 400 ml of cold phosphate buffer containing 30% sucrose. Serial frozen sections of L4–L5 DRGs, L1–L6 spinal cord segments and the medulla (15 or 60 μm in thickness) were reacted for the demonstration of peroxidase activity according to Mesulam (Mesulam, 1978) using 3,3',5,5'-tetramethylbenzidine (TMB) as chromogen, dehydrated in ethanol, cleared in xylene and mounted in Permount. Size-frequency distribution histograms of CTB-HRP-labelled neurons were generated by measuring the cross-sectional area (CSA) of neurons with clear-cut nuclei in representative serial sections of L5 DRGs of each animal by means of a light microscope equipped with a camera lucida and a digitizing tablet connected to a computerized system.

3.5. In situ hybridization

The synthesis of the cRNA probe and *in situ* hybridization were carried out as described by (Maniatis et al., 1982), with slight modifications. To generate TRPV1 gene-specific probes, total mRNA isolated from rat trigeminal ganglia was reverse transcribed using the universal dT17-adapter primer (5'-GACTCGAGTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3', M-MuLV reverse transcriptase; Fermentas, Vilnius, Lithuania) according to the manufacturer's recommendations. This cDNA template was used to perform RT-PCR with the following primer combination: forward 5'-AACCATGGAACAACGGGCTAGC-3'; reverse 5'-AACTCGAGTTAGAACAGAGCTGACA-3'. The amplified 255 bp length product was cloned into pcDNA3 vector (Invitrogen, Carlsbad, CA, USA). The identity of the amplified product was confirmed by DNA sequencing and Northern blotting. After

linearization of the vectors, sense and antisense digoxigenin-11-UTP-labelled cRNA probes were transcribed with T7 or SP6 polymerases, using a DIG RNA labelling kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol. For *in situ* hybridization, DRGs were quickly removed, embedded in Cryomatrix embedding material (Shandon Scientific, Pittsburgh, PA, USA) and frozen immediately at -70 ^{o}C. Serial frozen sections of DRGs (15 µm in thickness) were cut on a cryostat, and thaw-mounted onto 3-aminopropyltriethoxysilane-coated glass slides. Sections were air-dried and stored at -20 ^{o}C until further processing. In order to improve the antigen retrieval sections were heated with microwave irradiation (5 + 4 min in 0.01 M Na-citrate solution, pH 6), left in the same buffer for 10 min until the forthcoming steps (Yang et al., 1999, Rangell and Keller, 2000, Mitchell et al., 2001, Relf et al., 2002, Szigeti et al., 2003). The specimens were fixed for 5 min in 2x sodium chloride – sodium citrate (SSC) buffer (0.3 M NaCl and 0.03 M Na-citrate, pH 7.0) containing 4% formaldehyde, washed twice in 2x SSC buffer for 2 min, permeabilized with 0.1% Triton X-100, washed again as before, and then rinsed in 0.1 M triethanolamine containing 0.25% acetic anhydride at room temperature for 5 min. Hybridization was performed in 50 µl hybridization solution (50% formamide, 5x sodium chloride - sodium phosphate - EDTA buffer, 1x Denhardt's reagent, 10% dextran sulfate, 50 mM dithiothreitol, 100 µg/ml salmon sperm DNA and 100 µg/ml yeast tRNA containing 200 nmol/ml labelled probe) under parafilm cover slips in a humidified chamber at 56 °C for 20 h. The sections were extensively rinsed in 2x SSC buffer supplemented with 50% formamide at 50 °C for 15 min, treated with RNase A at 37 °C for 30 min, and washed again in 2x SSC – 50% formamide solution at 50 °C. To block nonspecific antibody binding, sections were incubated with Buffer 1 (100 mM Tris-HCl and 150 mM NaCl, pH 7.5) containing 5% normal goat serum for 1 h at room temperature, followed by incubation with alkaline phosphatase-conjugated anti-digoxigenin antibody (1:2500, Boehringer Mannheim GmbH, Mannheim, Germany) in Buffer 1 at 4 °C overnight. Sections were washed in Buffer 1 for 3×5 min, rinsed in Buffer 2 (100 mM Tris-HCl, 100 mM NaCl and 50 mM $MgCl_2$, pH 9.5) for 10 min and developed in Buffer 2 containing 340 µg/ml nitro blue tetrazolium and 180 µg/ml 5-bromo-4-chloro-3-indolyl phosphate for 12 h in a dark chamber. The reaction was terminated by rinsing the slides in a buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) for 10 min. The sections were covered with glycerol.

3.6. Quantitative RT-PCR measurements

To measure changes in the total TRPV1 mRNA expression in DRGs affected by the transection or capsaicin treatment of the sciatic nerve quantitative RT-PCR was used. Rats were terminally anaesthetized 3, 14 and 30 days after surgery and the L5 DRGs were excised and transferred into 1 ml ice-cold Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total mRNA was isolated by Trizol solution according to the protocol of the manufacturer. The extracted total mRNA was reverse transcribed by using BioRad iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Specific primers were designed to amplify TRPV1 and beta-2-microglobulin (B2-MG, reference gene) by using the Primer-Blast open source software (http://www.ncbi.nlm.nih.gov/tools/primer-blast). The sequences of the primers were as follows: B2-MG (NM_012512; reference gene): 5'-TCTCCGGTGGATGGCGAGAGT–3' (reverse); 5'-GCTCGCTCGGTGACCGTGATC-3' (forward); TRPV1 (NM_031982.1): 5'-TGTCTTCCGGGCAACGTCCA-3' (reverse); 5'-AAGCGCCTGACTGACAGCGA-3' (forward). Primers were synthesized by Integrated DNA Technologies (Leuven, Belgium). These primers produced distinct PCR amplification products with length of 129 bp for TRPV1 and 106 bp for B2-MG, as confirmed by gel-electrophoresis. Quantitative RT-PCR was performed in triplicates utilizing SYBR Green technique (iQ SYBR Green Supermix, Bio-Rad, Hercules, CA, USA) and BioRad MyiQ5 Real Time Detection System running the following amplification protocol: 10 minutes on 95ºC (hot start) followed by 40 amplification cycles (denaturation: 10 s on 95ºC, annealing: 30 s on 56ºC; elongation and detection: 20 s on 72ºC). At the end of the amplification melt-curve analysis was also applied to exclude non specific fluorescent signals. Relative changes of target (TRPV1) mRNAs as corrected with the housekeeping reference gene B2-MG were calculated by using the Pfaffl-method (Pfaffl, 2001).

3.7. TRPV1 immunohistochemistry

The animals were deeply anesthetized and perfused transcardially with an aldehyde fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The L5 DRG was removed and postfixed in the same fixative for 2 h, and then placed into a phosphate-buffered 30% sucrose solution. Representative serial sections of L5 DRGs 15 μm in thickness were cut

on a cryostat and mounted on gelatin-coated glass slides. Sections were rinsed twice in phosphate-buffered saline (PBS) and incubated overnight with the primary antibody (1:1000; rabbit anti-TRPV1 IgG, ACC030, Alomone Labs, Jerusalem, Israel) with 0.3% Triton X100 added. After rinsing in PBS, the sections were incubated for 2 h with the secondary antibody (1:500 biotin-conjugated donkey anti-rabbit IgG, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted in PBS containing 0.3% Triton X100. To visualize the biotin-conjugated antibody, the sections were rinsed and treated with the Vectastain ABC Elite staining kit (Vector laboratories, Burlingame, CA, USA) according to the instructions of the manufacturer. The sections were dehydrated and covered with DPX mounting medium (Fluka, Buchs, Switzerland).

3.8. Semiquantitative densitometry

The sections cut from the DRGs and processed for visualization of the TRPV1 mRNA by *in situ* hybridization or the TRPV1 protein by immunohistochemistry were examined under bright-field illumination with a DMLB microscope (Leica, Wetzlar, Germany) equipped with a Nikon Coolpix (Nikon, Japan) digital camera. Under identical conditions, microphotographs were taken of DRGs relating to control sciatic nerves and sciatic nerves transected or treated perineurally with capsaicin following a systemic random sampling method. The optical density of DRG neurons with clear-cut nuclei was measured by means of the NIH Scion Image analysis program. In sections processed for the demonstration of TRPV1 mRNA, many neurons exhibited granular staining of different intensities in the perikaryon. In contrast, in labelled neurons the TRPV1 immunoreactivity displayed diffuse staining throughout the cell bodies and sometimes in their axons. Gray values (GVs) between 0 and 255 were assigned to each neuron with a clearly visible nucleus and their CSAs were measured. Relative optical densities (RODs) were determined according to the equation $ROD = \log_{10} (255/ (255\text{-}GV))$. The CSA and ROD for each cell were determined and plotted as distribution histograms or scatter plots.

3.9. Classification of DRG neurons

The DRG neurons were classified into different subpopulations by using a statistical approach. Pilot experiments suggested the existence of 3 distinct neuronal subpopulations in the control DRGs, with different levels of mRNA signal and TRPV1 immunostaining. Using the ROD and CSA data discriminant analysis was performed to define the ROD classification effect among the different subpopulations of DRG neurons. To determine the threshold values of ROD providing the optimal sensitivity-specificity relations for the separation of the neuronal subpopulations, the receiver operating characteristic (ROC) method was applied pairwise (Armitage P, 2001, Armitage and Colton, 2005). Since ROC method describes the performance of the discrimination only between two groups, therefore data of two original groups were aggregated and compared with the remaining third original group. The results of the discriminant analysis were used for the selection of groups to be aggregated.

3.10. Western blot analysis

L5 DRGs were removed from rats 3, 14 and 30 days after perineural capsaicin treatment or transection of the sciatic nerves and were homogenized immediately in ice-cold Radio Immuno Precipitation Assay (RIPA) buffer containing 50 mM Tris (pH 8), 150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulphate (SDS), 2 μg/ml leupeptin (Sigma) and 1 μg/ml pepstatin (Sigma). The homogenates were centrifuged at 15000 g for 10 min. The pellet was discarded and protein concentrations from the supernatant were determined according to the method of (Lowry et al., 1951). Protein samples (60 μg/well) were separated through a 12% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and blocked for 12 h in 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20. The membranes were incubated for 2 h with rabbit anti-TRPV1 (1:500, Chemicon, Temecula, CA, USA) and mouse anti-β-actin primary antibody (1:20000, Santa Cruz Biotechnology, Santa Cruz, California, USA) in 1% nonfat dry milk in 0.1% TBS–Tween 20. After three washes in 0.1% TBS-Tween 20, the membranes were incubated for 1 h with the appropriate peroxidase conjugated secondary antibodies (1:2000, Jackson ImmunoResearch Europe Ltd. Cambridgeshire, UK), and washed five times as before. The enhanced chemiluminescence method (ECL Plus Western blotting detection reagent; Amersham Biosciences Little Chalfont, Buckinghamshire, UK) was used to reveal

immunoreactive bands according to the manufacturer's protocol. The films were scanned at 600×600 dpi resolution and the densitometric quantification was performed by the ImageJ public domain image processing and analysis software (NIH, Bethesda, MD, USA). After subtracting background, TRPV1 band densities were normalized to β-actin. The ratio of the TRPV1 to β-actin band density was used to calculate the changes in TRPV1 expression. Results of three independent experiments are shown as means ± SD.

3.11. Statistical analysis

The experimental data are shown as means ± S.D. Statistical analyses were performed with ANOVA and Holm-Sidak, Brown-Forsythe or Bonferroni correction methods for post hoc comparisons by using SPSS (v.18, Statistical Software package, IBM Corporation, NY, USA). Differences between groups were considered statistically significant if $p < 0.05$.

4. RESULTS

4.1. Effect of neonatal capsaicin treatment on the lesion-induced alterations in the spinal distribution of myelinated primary afferent fibres

The injection of CTB-HRP into an intact nerve resulted in the labelling of the deeper layers of the spinal dorsal horn, but not the substantia gelatinosa (Fig. 2A). However, after the injection of the tracer into a chronically transected nerve, heavy homogeneous peroxidase staining could be detected not only in the deep dorsal horn, but also in the substantia gelatinosa and the marginal zone (Fig. 2B).The injection of CTB-HRP into the intact sciatic nerve of the capsaicin-pretreated rats resulted solely in the labelling of the deeper layers of the dorsal horn, the substantia gelatinosa remained free of labelling (Fig. 2C). The injection of the tracer into the chronically transected nerve of the capsaicin-pretreated rats resulted in a strong labelling of the deep dorsal horn and also a faint, but distinct labelling of the substantia gelatinosa (Fig. 2D). This latter labelling was confined to a few individual nerve fibres and was much weaker than the essentially homogeneous strong labelling seen after nerve transection in the control animals (Fig. 2B). In the medulla, labelling was observed in the gracile nucleus relating to both the intact and the transected nerves. The intensity and extent of the labelling was increased ipsilaterally to the injured nerve (Fig. 2E). This lesion-induced increase in labelling was also present in the capsaicin-pretreated rats (Fig. 2F).

In control rats, light microscopy of the L5 spinal ganglion and analysis of the size-frequency distribution histograms revealed that, the CTB-HRP-labelled neurons in the ganglia relating to the intact sciatic nerve involved mostly larger ganglion cells, although a moderate proportion of small cells were also labelled (Figs. 3A and 4a). In contrast, after nerve transection, a majority of the small cells displayed peroxidase activity, indicating the presence of CTB-HRP (Figs. 3B and 4b). Neonatal treatment with capsaicin resulted in a profound reduction in the proportion of small dorsal root ganglion neurons. In the ganglia relating to the intact nerve in these rats, CTB-HRP was localized to larger cells (Figs. 3C and 4c). In the ganglia relating to the transected nerve, an increase in the proportion of labelled cells of all sizes was observed (Figs. 3D and 4d).

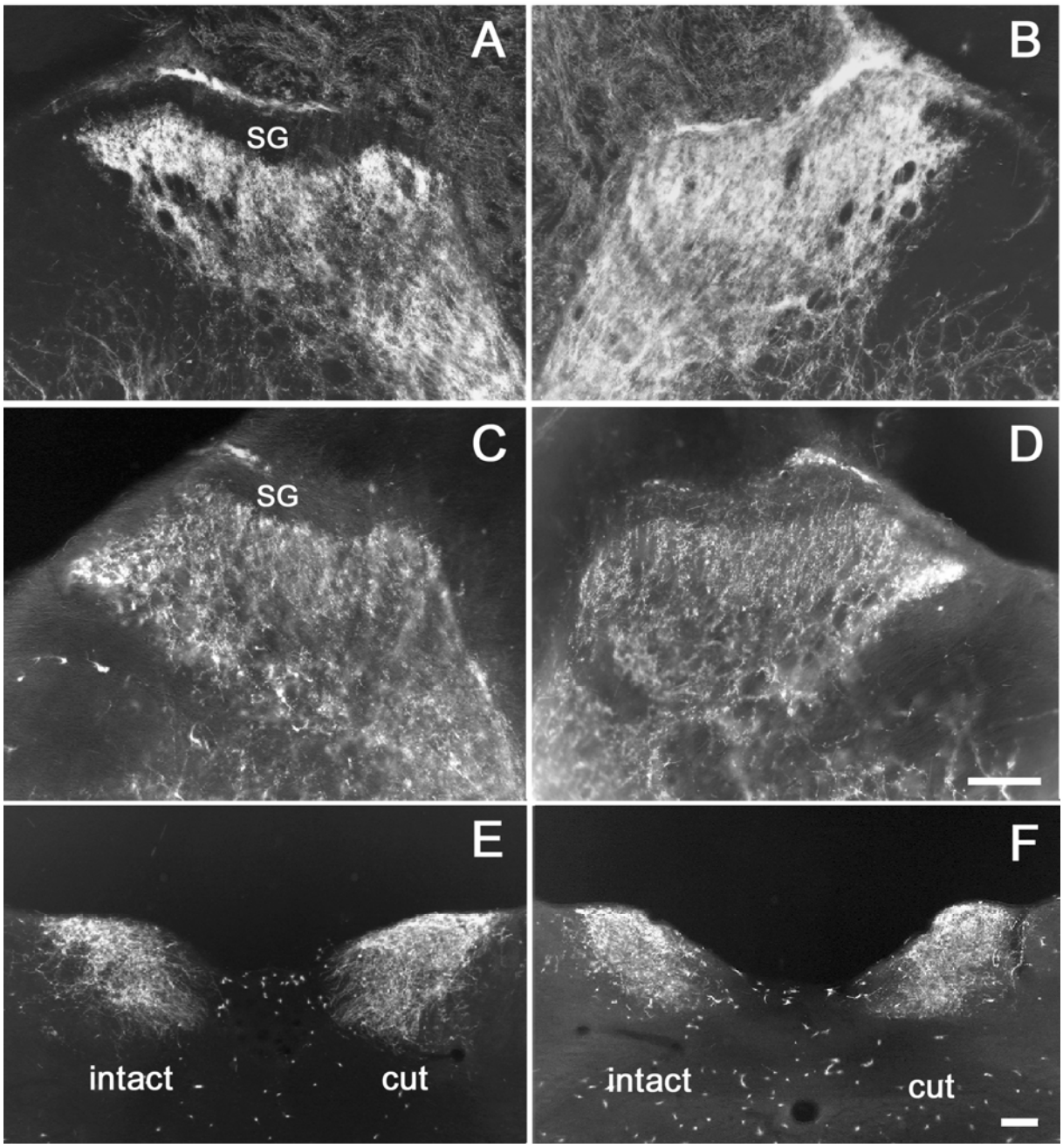


Fig. 2. Inverse microphotographs illustrating the distribution of spinal primary afferents transganglionically labeled with CTB-HRP in the spinal dorsal horn and the medulla oblongata relating to the intact (A, C) and the transected (B, D) sciatic nerves of the control (A, B, E) and the capsaicin pretreated (C, D, F) rats. SG = substantia gelatinosa. The scale bars in D and F correspond to 100 μm and apply to A–D and E–F, respectively.

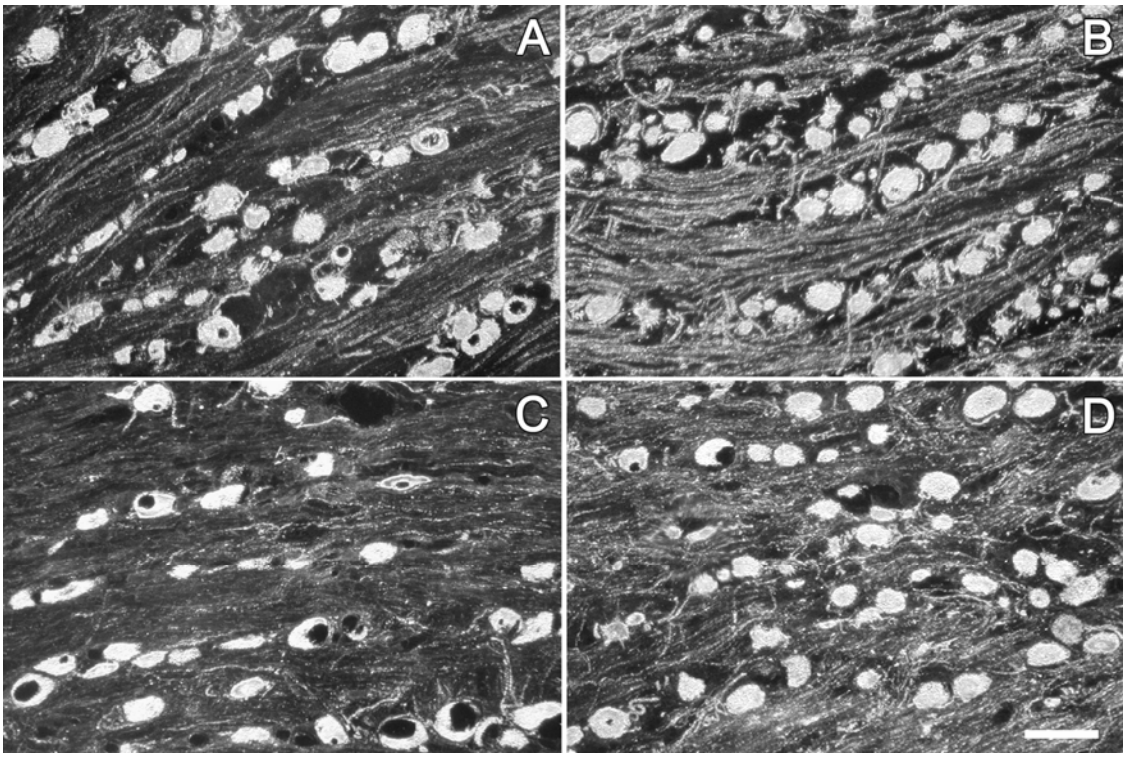


Fig. 3. Inverse microphotographs showing CTB-HRP-labeled neurons of spinal ganglion L5 relating to the intact (A, C) and the transected (B, D) sciatic nerves of the control (A, B) and the capsaicin-pretreated rats (C,D). Note the increase in number of the labeled small cells after nerve transection in the control (B), but not in the capsaicin-pretreated (D) rats. Scale bar = 100 μm and applies to all microphotographs

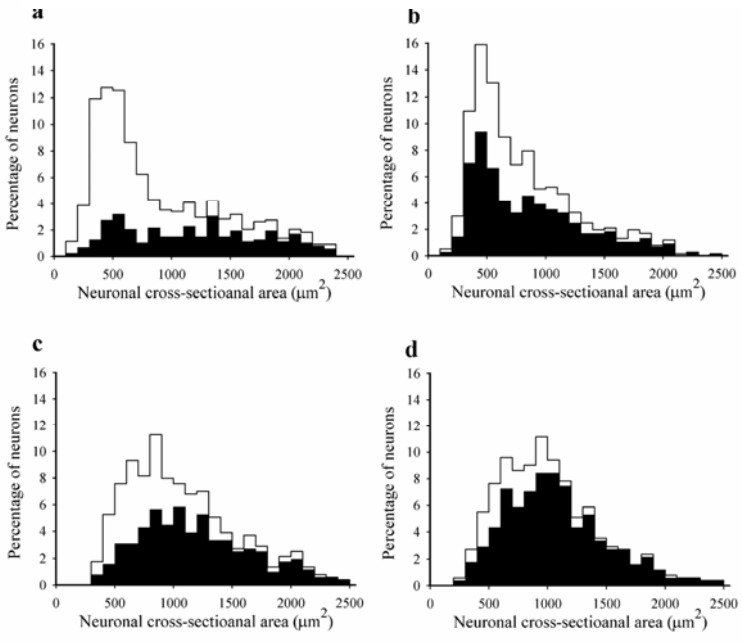


Fig. 4 Size-frequency distribution histograms of neuronal populations of DRGs relating to intact (a, c) and transected (b, d) sciatic nerve of control (a, b) and capsaicin-pretreated (c, d) rats. Clear histograms represent the total neuronal population, whereas filled histograms represent the CTB-HRP-labeled neurons.

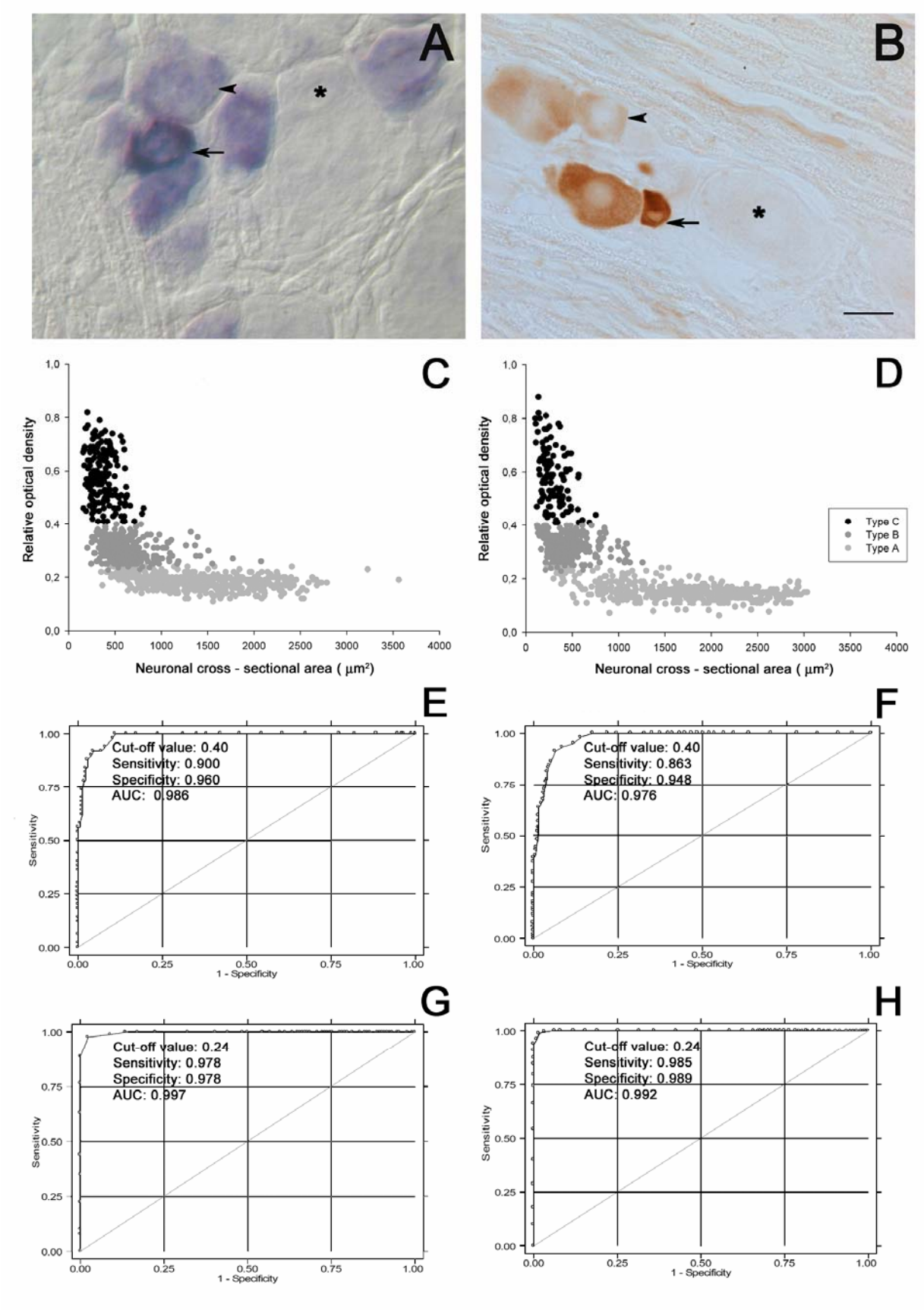
4.2. Localization of TRPV1 mRNA and protein in the L5 DRG of the rat

In the control DRGs three types of neurons could be distinguished, with different levels of TRPV1 mRNA expression and TRPV1-immunostaining. Small to medium-sized neurons displayed intense and moderate expression levels, whereas the larger neurons were mostly devoid of TRPV1 mRNA and protein (Fig. 5A-D). Since the discriminant analysis showed the best separation of group C in respect of the optical densities of the cells, therefore the ROD values of this group were compared with the aggregated data of the original B and A groups. The optimal cut-off point for the TRPV1 mRNA ROD to distinguish between group C and the remaining population was 0.40, which provided a specificity of 96% and a sensitivity of 90%. Similarly, a cut-off value of 0.24 provided the optimal differentiation between groups A and B (Fig. 5E, G). ROC analysis using CSA data of group A and the aggregated B+C groups resulted in a cut-off value of 1000 μm^2 providing specificity of 91% and sensitivity of 82%. Since these lower values indicated an inferior discrimination performance of CSA as compared to ROD, therefore the ROD values and the calculated cut-off points for ROD were used throughout in our experiments to identify different neuronal populations in DRGs related either to control or treated nerves.

Type C and B neurons were characterized by their small (CSA range: 0-400 μm^2) and medium sizes (range: 410-900 μm^2) and high (0.41-1) and moderate (0.25-0.40) RODs, respectively. The population of type A neurons comprised cells of various sizes with low RODs (0-0.24) which hardly exceeded the background ROD. The type C and B neurons were regarded as expressing high and moderate levels of TRPV1 mRNA, whereas type A cells were classified as TRPV1-negative neurons. The *in situ* hybridization experiments revealed that around half of the DRG cells expressed TRPV1 mRNA in control ganglia. The type C cells accounted for around 19% and the type B cells approximately 29% of the total neuronal population. About half (51%) of the cells in the DRGs were clearly negative for TRPV1 mRNA. Although the majority of the TRPV1 mRNA-negative neurons were large, some small neurons also exhibited low RODs.

Statistical analysis of the TRPV1-immunopositive neurons revealed three subpopulations of DRG neurons with respect to their TRPV1 protein content (Fig. 5F, H): the type C and B neurons were mainly small to medium-sized, with strong or moderate staining intensity, respectively, while the TRPV1-negative neurons were mostly large in size.

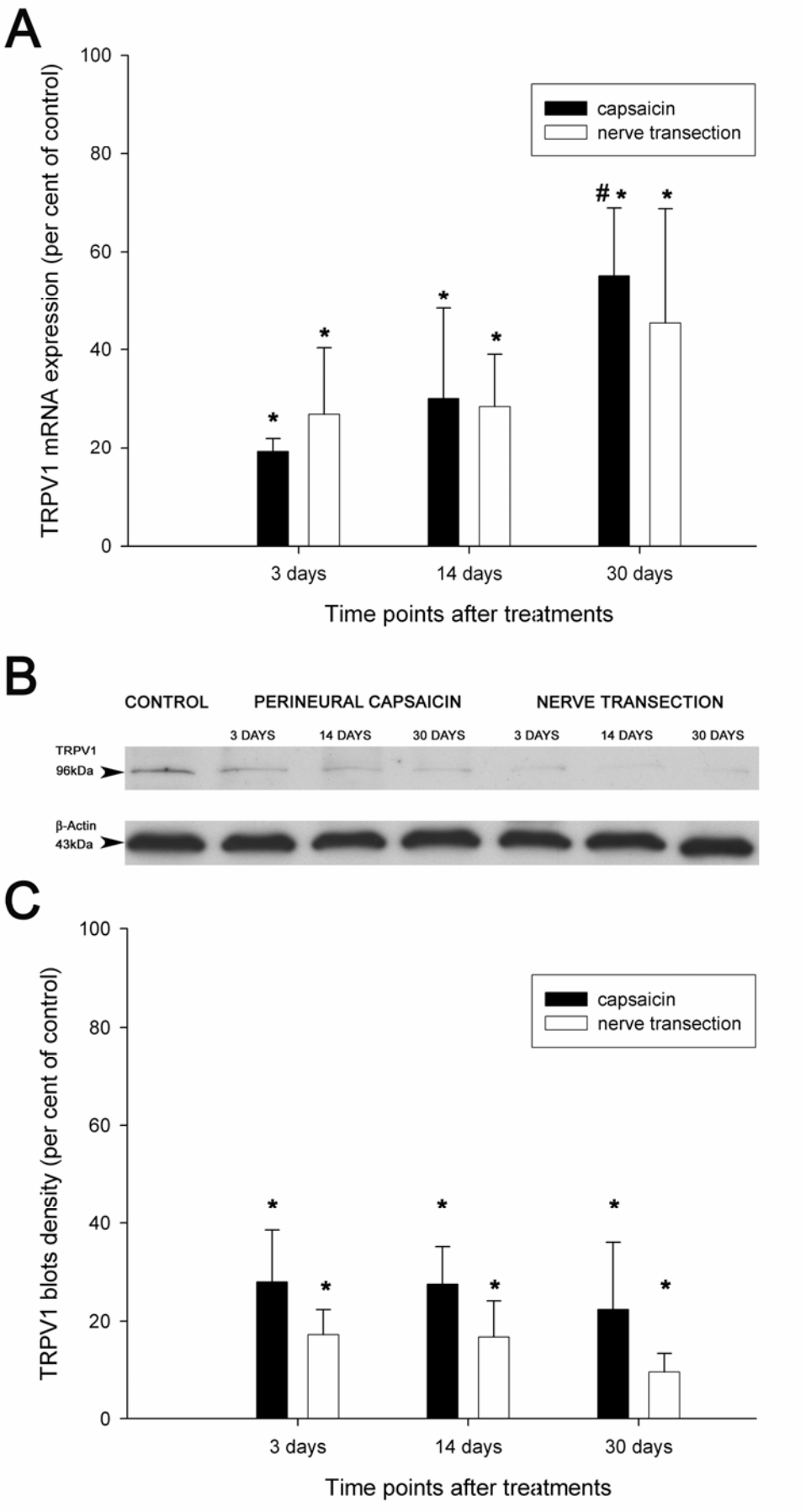
Fig. 5. A, B: In control ganglia, *in situ* hybridization (A) and immunohistochemistry (B) revealed small to medium-sized neurons with intense (arrows) and moderate (arrowheads) levels of TRPV1 mRNA and protein, respectively. Larger neurons were usually devoid of both TRPV1 mRNA and protein (asterisks). Scale bar indicates 25 μm. C, D: Scatter plots of DRG cells, showing the cell sizes and the three separate populations of neurons with intense, moderate and very low RODs. E, G: ROC analysis of TRPV1 mRNA RODs revealed the cut-off values for the separation of type C and B (E) and type B and A (G) neurons, respectively, and disclosed the high sensitivity and specificity of the analysis involving the use of ROD. F, H: ROC analysis of the RODs of TRPV1-immunopositive neurons revealed the cut-off values for the separation of type C and B (F) and type B and A (H) neurons, respectively, and disclosed the high sensitivity and specificity of the analysis using ROD.


A
B
C
Relative optical density
Neuronal cross - sectional area (μm²)
D
Relative optical density
Neuronal cross - sectional area (μm²)
Type C
Type B
Type A
E
Cut-off value: 0.40
Sensitivity: 0.900
Specificity: 0.960
AUC: 0.986
Sensitivity
1 - Specificity
F
Cut-off value: 0.40
Sensitivity: 0.863
Specificity: 0.948
AUC: 0.976
Sensitivity
1 - Specificity
G
Cut-off value: 0.24
Sensitivity: 0.978
Specificity: 0.978
AUC: 0.997
Sensitivity
1 - Specificity
H
Cut-off value: 0.24
Sensitivity: 0.985
Specificity: 0.989
AUC: 0.992
Sensitivity
1 - Specificity

4.3. Effects of perineural capsaicin treatment or transection of the sciatic nerve on the expression of the TRPV1 mRNA in the L5 DRG of the rat.

In the rat, the sensory fibres of the sciatic nerve originate from the fourth, the fifth and (to a much lower extent) the sixth lumbar DRGs (Green, 1968). Up to 85% of the neurons in the fifth lumbar DRG project their axons into the sciatic nerve (Yip et al., 1984, Aldskogius et al., 1988). In the present study, therefore, the fifth lumbar DRG was chosen to study possible changes in the expression of the TRPV1 receptor following two types of nerve injury: nerve transection, a physical injury resulting in neurotmesis, damage to all types of axons of the sciatic nerve (Seddon, 1943), and perineural treatment with capsaicin, which produces a selective chemodenervation of C-fibre afferents, but leaves the continuity of the nerve intact.
The experiments using quantitative RT-PCR showed an early and marked reduction in TRPV1 mRNA expression already 3 days after perineural capsaicin treatment. However, at later survival times quantitative RT-PCR measurements revealed a clear-cut tendency to recovery towards control expression levels resulting in a marked and statistically significant increase in TRPV1 mRNA at 30 days (Fig. 6A).

Fig. 6**.** Quantitative RT-PCR and Western blot analyses of the TRPV1 mRNA and protein expression. A: Results of 3-6 independent experiments demonstrate the time course of changes in TRPV1 mRNA expression measured with quantitative RT-PCR in L5 DRGs following perineural capsaicin treatment and transection of the sciatic nerve. Note the marked time-dependent increase in TRPV1 mRNA expression following perineural capsaicin treatment. B: Representative immunoblots of TRPV1 and β-actin proteins in L5 DRGs 3, 14 and 30 days after perineural capsaicin treatment and transection of sciatic nerve. C: Results of three independent experiments demonstrate the time course of changes in TRPV1 protein. Note the marked decreases in the TRPV1 protein at all time points after perineural capsaicin treatment and nerve transection. * : Significantly different from the control, $p < 0.05$. #: Significantly different from the 3-day value, $p < 0.05$.

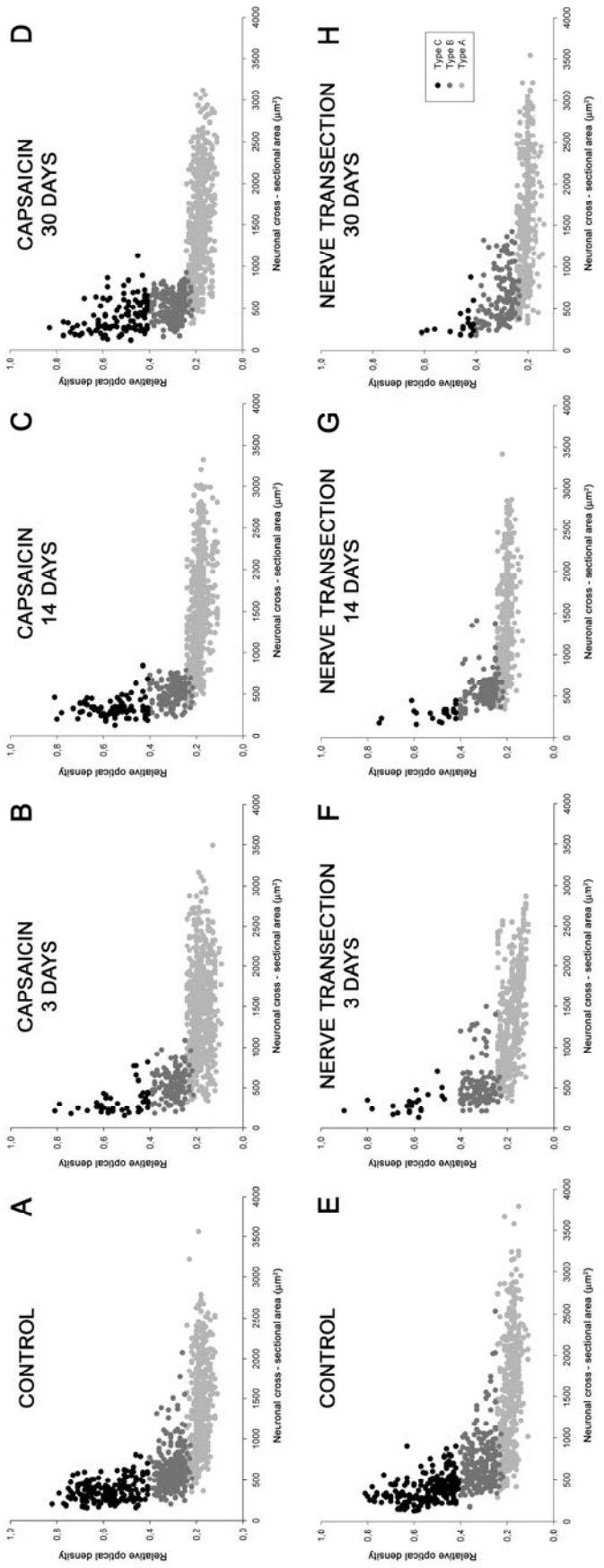

A
TRPV1 mRNA expression (per cent of control)
100
80
60
40
20
0
capsaicin
nerve transection
#
*
3 days
14 days
30 days
Time points after treatments
B
CONTROL
PERINEURAL CAPSAICIN
NERVE TRANSECTION
3 DAYS
14 DAYS
30 DAYS
3 DAYS
14 DAYS
30 DAYS
TRPV1
96kDa
β-Actin
43kDa
C
TRPV1 blots density (per cent of control)
100
80
60
40
20
0
capsaicin
nerve transection
*
3 days
14 days
30 days
Time points after treatments

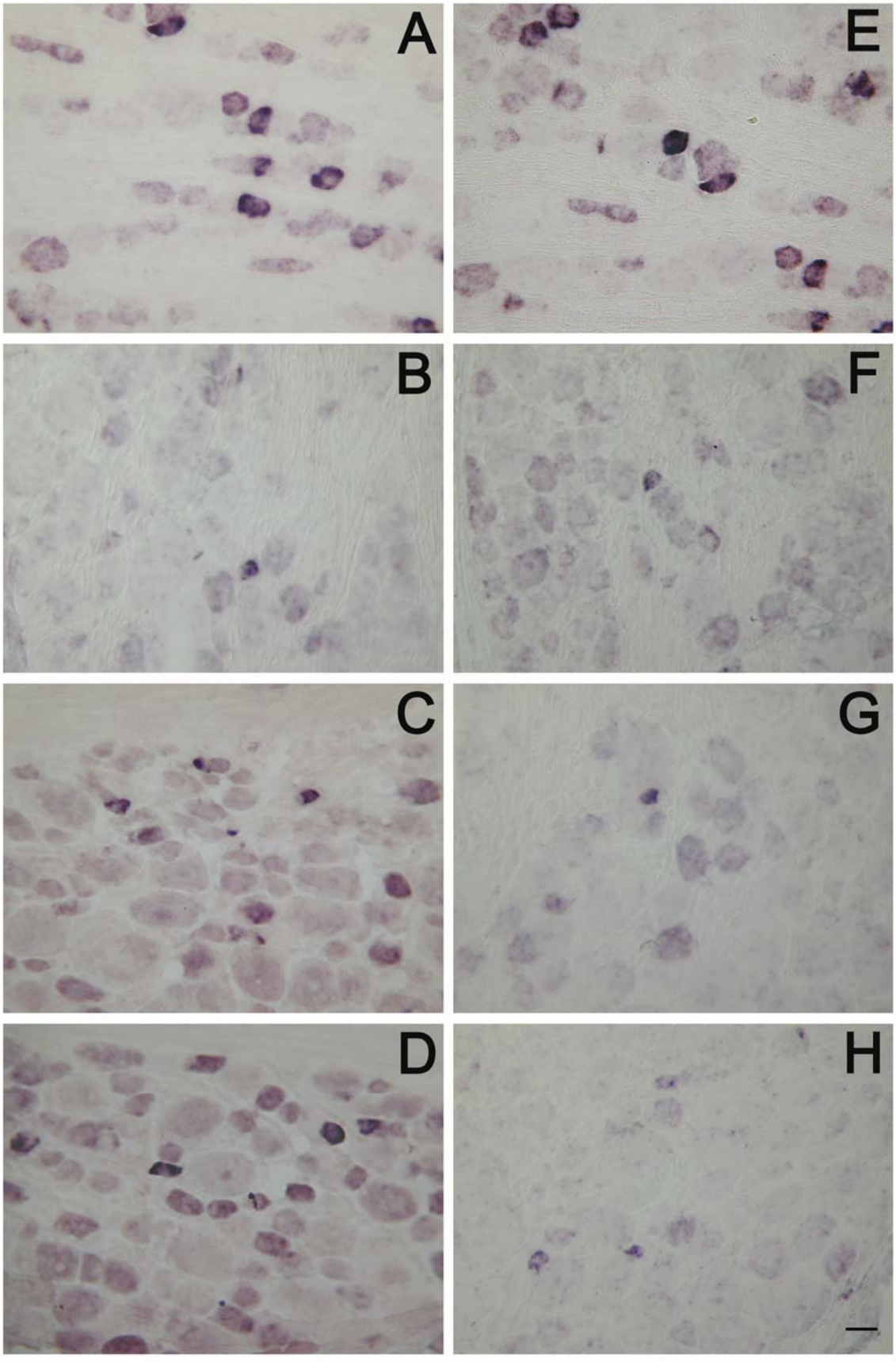
In situ hybridization analysis confirmed these findings by showing a rapid decrease in the expression of TRPV1 mRNA in the neurons of the fifth lumbar DRG, with reductions by about 50% and 75% in type B and C cells 3 days after the treatment (Figs. 7B and 8B). However, this initial decrease in the TRPV1 expression was followed by a distinct recovery and the proportion of TRPV1 mRNA-expressing neurons gradually increased up to 70% of the control levels toward the end of the study (Figs 7D and 8D).

Similarly to perineural treatment with capsaicin, peripheral nerve transection resulted in rapid and marked reductions in TRPV1 mRNA expression in the type B and C cells of the related fifth lumbar DRG 3 days after surgery (Figs. 7F and 8F). However, in contrast with capsaicin treatment, there was no recovery in the TRPV1 mRNA expression after peripheral nerve transection, it remained at a low level for the entire remainder of the study period (Figs. 7H and 8H). In accordance with these findings obtained with in situ hybridization, quantitative RT-PCR measurements revealed marked and significant reductions in the TRPV1 mRNA expression 3 and 14 days after nerve transection. TRPV1 mRNA expression showed some increase after 30 days, but that did not reach significance (Fig. 6A). The percentage distributions of each cell populations at all time points after the two different treatments are summarised in Table 1.

Fig. 7 Scatter plots showing the time course of changes in the populations of TRPV1 mRNA-expressing L5 DRG neurons following perineural capsaicin treatment and transection of the ipsilateral sciatic nerve. Symbols of decreasing graytone intensities denote type C, B and A neurons, respectively.

Fig. 8 Effects of perineural capsaicin treatment (A-D) or sciatic nerve transection (E-H) on the TRPV1 mRNA-expressing neurons in the L5 DRGs. Representative microphotographs illustrating the control DRGs (A, E), and the ganglia 3 days (B, F), 14 days (C, G) and 30 days (D, H) postoperatively. Scale bar indicates 20μm and applies to all microphotographs.


CONTROL
A
CAPSAICIN
3 DAYS
B
CAPSAICIN
14 DAYS
C
CAPSAICIN
30 DAYS
D
CONTROL
E
NERVE TRANSECTION
3 DAYS
F
NERVE TRANSECTION
14 DAYS
G
NERVE TRANSECTION
30 DAYS
H
Type C
Type B
Type A
Relative optical density
Neuronal cross - sectional area (µm²)


A
E
B
F
C
G
D
H

PERCENTAGE DISTRIBUTION OF TRPV1-EXPRESSING (B, C) AND TRPV1-NEGATIVE (A) L5 DRG CELL POPULATIONS 3, 14 AND 30 DAYS AFTER PERINEURAL CAPSAICIN TREATMENT AND NERVE TRANSECTION

PERINEURAL CAPSAICIN

	TRPV1 mRNA expression				TRPV1 immunohistochemistry			
Neuron type	**Control**	**3 days**	**14 days**	**30 days**	**Control**	**3 days**	**14 days**	**30 days**
C	19 ± 1.28	5 ± 0.74*	9 ± 0.62*#	12 ± 1.4*#	17 ± 1.73	2 ± 0.56*	4 ± 0.30*	3 ± 0.43*
B	29 ± 2.33	15 ± 1.03*	15 ± 0.91*	20 ± 1.2*#	36 ± 1.00	9 ± 2.23*	11 ± 0.72*	12 ± 0.82*
A	51 ± 3.13	79 ± 1.76*	75 ± 1.51*	69 ± 4.7*#	46 ± 1.00	89 ± 2.06*	84 ± 0.45*	84 ± 0.44*

NERVE TRANSECTION

	TRPV1 mRNA expression				TRPV1 immunohistochemistry			
Neuron type	**Control**	**3 days**	**14 days**	**30 days**	**Control**	**3 days**	**14 days**	**30 days**
C	18 ± 1.37	3 ± 0.36*	2 ± 0.03*	2 ± 0.20*	15 ± 1.73	2 ± 0.60*	5 ± 1.03*	4 ± 0.26*
B	28 ± 3.10	15 ± 1.03*	16 ± 1.72*	16 ± 1.46*	37 ± 4.70	16 ± 1.90*	16 ± 1.20*	18 ± 0 65*
A	53 ± 1.85	82 ± 1.33*	82 ± 1.69*	81 ± 1.26*	50 ± 0.60	80 ± 2.35*	78 ± 0.80*	77 ± 1 00*

Table 1 Data are expressed as means ± S.D. * significantly different from the control, $p < 0.05$. # significantly different from the 3-day value, $p < 0.05$.

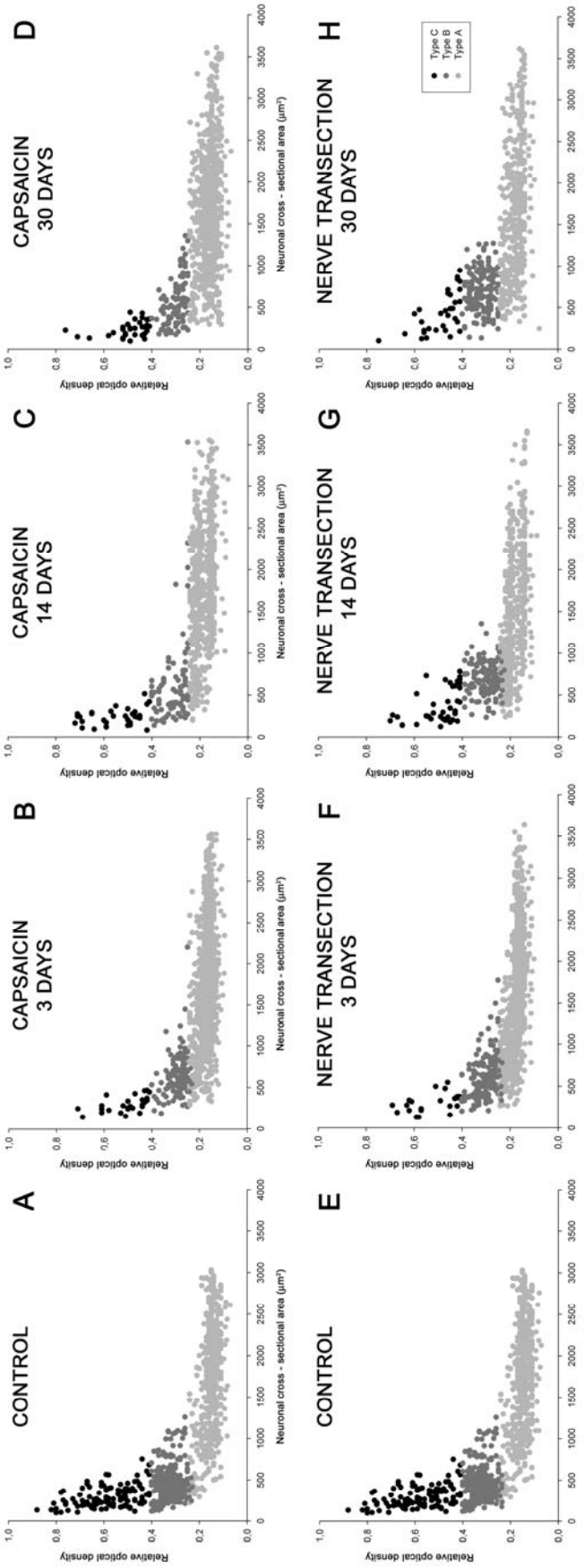
4.4. Effects of perineural capsaicin treatment or transection of the sciatic nerve on the expression of the TRPV1 protein in the L5 DRG of the rat

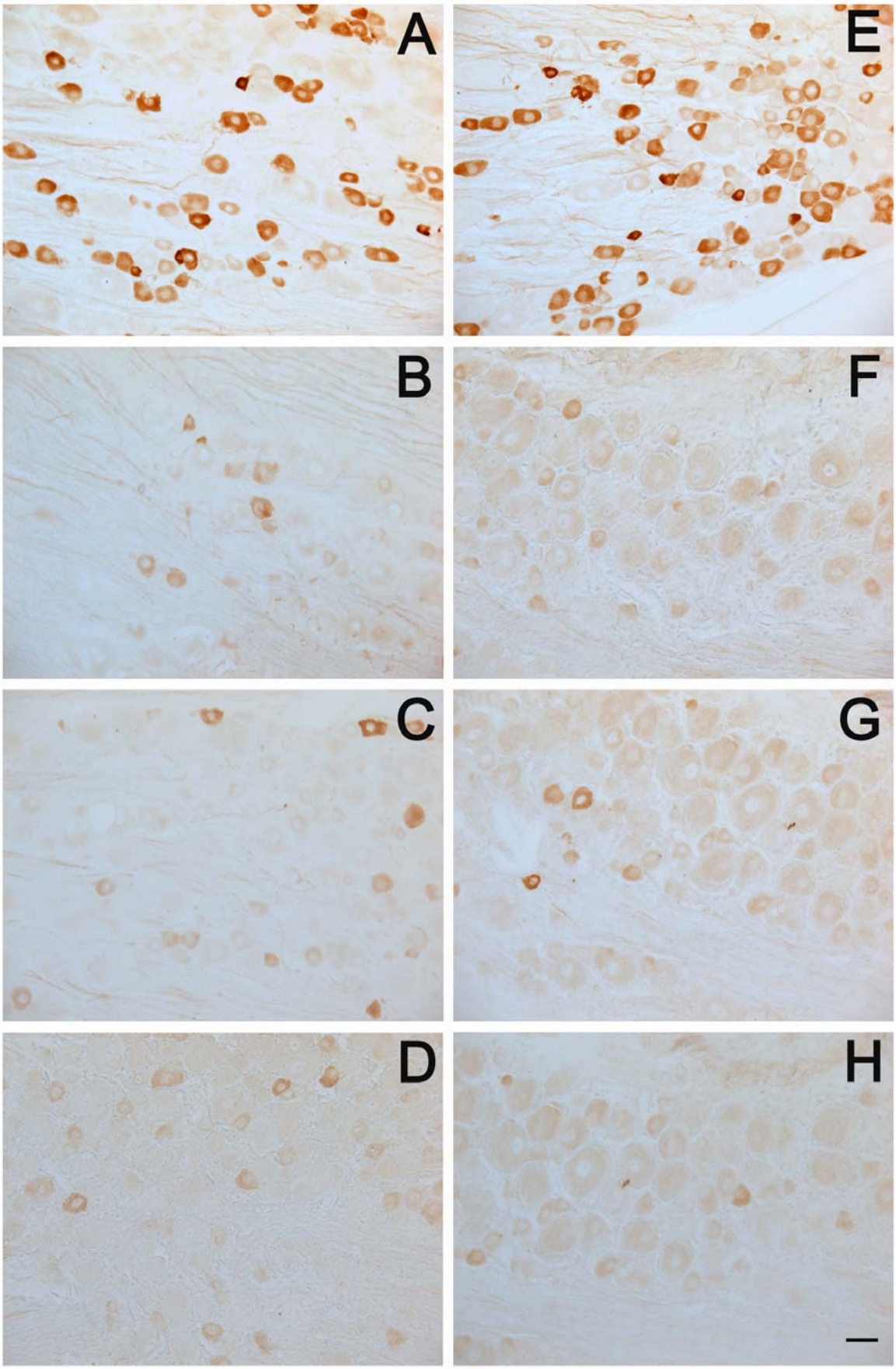
Study of the localization of the TRPV1 protein by means of immunohistochemistry revealed that the proportion of TRPV1-positive ganglion cells had decreased markedly (to about 30% of the control level) in the type B and C cells of the related fifth lumbar DRG 3 days after perineural capsaicin treatment (Figs 9B and 10B), and it remained at that low level throughout the entire period of the study (Table 1). The reduction in the proportion of type C cells was especially pronounced, by about 85%. Western blot analysis of the TRPV1 protein supported the immunohistochemical findigs. The TRPV1 protein was markedly and significantly reduced at all time points after perineural treatment with capsaicin or peripheral nerve transection (Figs 6B and 6C).

The decreases in TRPV1 mRNA expression and TRPV1 protein (by about 80%) were especially marked in the type C cells. The analysis of the experimental data clearly showed the time-dependent and cell type-specific changes in the expression of TRPV1 mRNA and protein, respectively. Note the tendency of the recovery of mRNA expression following perineural capsaicin treatment as compared to the permanently suppressed expression following axotomy (Fig. 11A). Similar recovery can not be observed in case of direct detection of the TRPV1 protein by immunohystochemistry (Fig. 11B)

Fig. 9 Scatter plots showing the time course of changes in the populations of TRPV1-immunoreactive L5 DRG neurons following perineural capsaicin treatment and transection of the ipsilateral sciatic nerve. Symbols of decreasing graytone intensities denote type C, B and A neurons, respectively.

Fig. 10 Effects of perineural capsaicin treatment (A-D) or sciatic nerve transection (E-H) on the TRPV1 protein content of the neurons in the L5 DRGs. Representative microphotographs illustrating the control DRGs (A, E), and the ganglia 3 days (B, F), 14 days (C, G) and 30 days (D, H) postoperatively. Scale bar indicates 40µm and applies to all microphotographs.


A
CONTROL
B
CAPSAICIN
3 DAYS
C
CAPSAICIN
14 DAYS
D
CAPSAICIN
30 DAYS
E
CONTROL
F
NERVE TRANSECTION
3 DAYS
G
NERVE TRANSECTION
14 DAYS
H
NERVE TRANSECTION
30 DAYS
Type C
Type B
Type A
Relative optical density
Neuronal cross - sectional area (µm²)


A
E
B
F
C
G
D
H

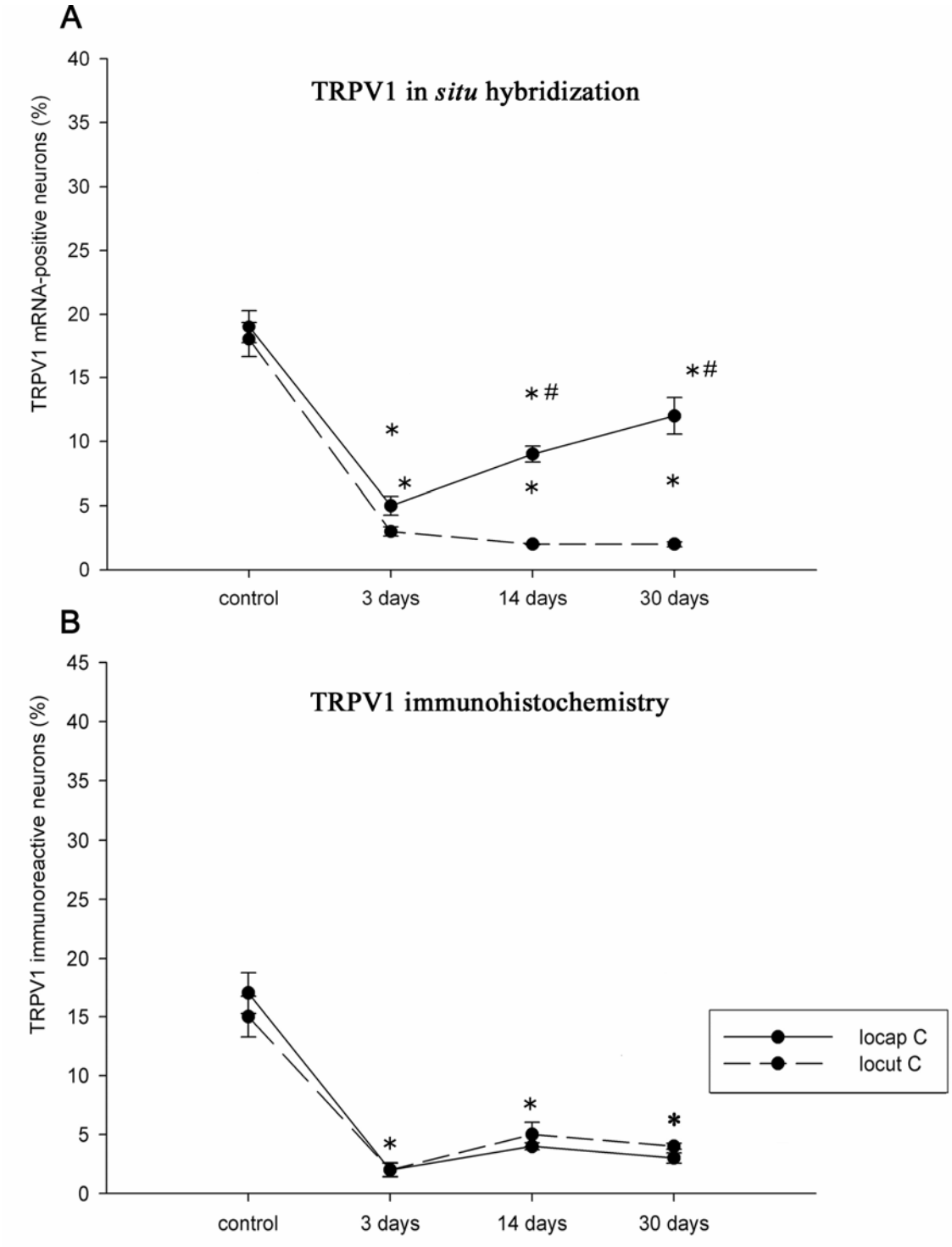


Fig. 11 Time course of the changes in the expression of TRPV1 mRNA (A) and the TRPV1 protein (B) in type C primary sensory neurons. Data represent the relative proportions of the type C neurons following perineural capsaicin (locap) or peripheral nerve transection (locut). *: significantly different with the corresponding control value; #: significantly different from the 3 days value.

5. DISCUSSION

Peripheral nerve transection, classified as neurotmesis (Seddon, 1943), results in complete severance of the nerve producing not only local structural and functional changes, but also profound transganglionic changes in the affected axon terminals of the spinal dorsal horn (Grant and Arvidsson, 1975, Grant and Ygge, 1981, Aldskogius et al., 1985, Jancsó, 1992). The substantia gelatinosa is the principal projection territory of thin primary afferents (Szentágothai, 1964) the majority of which are chemosensitive C-fibre nociceptive primary afferents (Jancsó and Knyihár, 1975, Jancsó et al., 1980b, Ritter and Dinh, 1988, Jancsó and Lawson, 1990, Jancsó, 1992, Guo et al., 1999, Hiura, 2000). Changes in C-fibre function and/or morphology have been implicated in the mechanisms of neuroplastic alterations and modulation of neuronal connectivity in the spinal dorsal horn (Wall and Fitzgerald, 1982). Structural changes involving transganglionic degeneration of C-fibre spinal primary afferent fibres have been shown to occur after peripheral nerve lesions (Jancsó and Lawson, 1990, Jancsó, 1992).

In the present study, the distribution of primary afferent fibres in the spinal dorsal horn was investigated through the injection of CTB-HRP into the chronically transected sciatic nerve. CTB, which is responsible for the binding of the toxic A subunit of choleratoxin to its receptor, the GM1 ganglioside, has been shown to specifically label the large, type A ganglion cells in the rat that give rise to myelinated axons (Robertson and Grant, 1989). Our findings confirmed previous reports showing the transganglionic labelling of the substantia gelatinosa after the injection of CTB-HRP into injured, but not into intact peripheral nerves (Robertson and Grant, 1985, Woolf et al., 1995, Lekan et al., 1996, Nakamura and Myers, 1999, Bao et al., 2002, Sántha and Jancsó, 2003). We also demonstrated that neonatal capsaicin treatment produced irreversible loss of small sensory ganglion neurons, and consequently a selective C-fibre deafferentation of the spinal dorsal horn. Importantly, neonatal capsaicin treatment prevented the heavy labelling by CTB of the substantia gelatinosa following a peripheral nerve lesion. Previous electron microscopic histochemical studies furnished direct evidence for the transport of choleragenoid by unmyelinated axons in the dorsal roots axons relating to a transected, but not to an intact sciatic nerve (Sántha and Jancsó, 2003). In capsaicin-pretreated rats, nerve transection induced an increase in the proportion of CTB-HRP labelled

large dorsal root ganglion neurons, which suggests that the increased uptake and transport of CTB-HRP by the myelinated afferents is apparently independent of C-fibre function/integrity. Finally, the increase in the intensity and extent of the choleragenoid labelling of the gracile nucleus indicates that an injury-induced increase in the labelling of the A-fibres occurs also in the absence of C-fibres. These results, by showing an almost complete abolition of the injury-induced transganglionic labelling of the substantia gelatinosa in rats treated neonatally with capsaicin furnished further evidence for the pivotal role of chemosensitive primary afferent neurons in the apparent structural changes which develop after peripheral nerve lesions (Jancsó et al., 2004). The findings provide further support for the view that lesion-induced transganglionic labelling of the substantia gelatinosa may be accounted for by a phenotypic switch of C-fibre primary afferent neurons rather than a sprouting response of myelinated dorsal root fibres (Sántha and Jancsó, 2003). Hence, a peripheral nerve lesion induces fundamental changes in the chemical phenotype of chemosensitive primary sensory neurons, which express the capsaicin/TRPV1 receptor: these neurons which, in the intact animal do not bind the GM1 ganglioside change their phenotype and, similarly to large DRG neurons, turn into GM1 binding ganglion cells. GM1 plays a critical role in the mechanisms of the trophic actions of growth factors, in particular nerve growth factor (NGF, (Schwartz and Spirman, 1982, Leon et al., 1984, Mutoh et al., 1998)which, in turn, plays a pivotal role in the regulation of the capsaicin sensitivity of DRG neurons (Winter et al., 1988). Since in rat sensory ganglion neurons the availability of NGF, and consequently the expression of the capsaicin/TRPV1 receptor is critically dependent on the retrograde axonal transport (Winter et al., 1988, Aguayo and White, 1992, Bevan and Winter, 1995), it seemed worthwhile to initiate further studies to reveal possible changes in the expression of the TRPV1 receptor following different types of peripheral nerve lesions. Two different types of peripheral nerve injuries were choosen: nerve transection resulting in neurotmesis (Seddon, 1943) and affecting all types of axons running in the affected nerve, and perineural capsaicin treatment which produces a selective chemodenervation of C-fibre primary afferent fibres and selective regional analgesia, i.e. loss of chemogenic pain sensation and reduction of nociceptive responses elicited by noxious thermal and chemical stimuli (Jancsó et al., 1980a, 1987, Fitzgerald and Woolf, 1982, Gamse et al., 1982, Jancsó and Lawson, 1990).

Chemosensitive primary sensory neurons which express the TRPV1 receptor play a fundamental role in the transmission of nociceptive impulses (Jancsó et al., 1977, Caterina et

al., 1997, Julius and Basbaum, 2001). The level of expression of the TRPV1 receptor is an important determinant of the nociceptor function. Increases in TRPV1mRNA expression and in peripherally directed axonal transport of TRPV1 protein have been demonstrated to be associated with neuropathic pain states and inflammation (Tohda et al., 2001). Conversely, knock-down of the TRPV1 gene prevents the development of inflammatory hyperalgesia in the rat (Caterina et al., 2000, Davis et al., 2000, Kasama et al., 2007). Hence, TRPV1 receptor antagonism or procedures which inhibit the activation of the receptor may produce significant antinociception. Indeed, local application of capsaicin and some other vanilloids directly onto peripheral nerve trunks has been shown to provide long-lasting and selective chemical and thermal analgesia, confined to the region innervated by the affected nerve (Jancsó et al., 1980, Gamse et al., 1982, Fitzgerald, 1983, Kissin et al., 2002, Knotkova et al., 2008, Jancsó et al., 2011). Despite numerous investigations that have made use of perineural capsaicin treatment (Gamse et al., 1982, Gibson et al., 1982, Chung et al., 1985, Jancsó and Lawson, 1987, 1990, Jancsó et al., 1987, Pini et al., 1990, Jancsó and Ambrus, 1994, Kissin et al., 2002), the mechanism of analgesia induced by perineural capsaicin remained unclear.

In the present experiments, cell size and the ROD of the mRNA signal and of the TRPV1 immunostaining were measured and a statistical approach was applied to classify subpopulations of DRG neurons which express the TRPV1 receptor. The results of the semi-quantitative morphometric analysis, *in situ* hybridization, RT-PCR, immunohistochemistry and Western blot analysis confirmed and extended previous reports (Jancsó and Lawson, 1990, Helliwell et al., 1998, Shi et al., 2001, Bridges et al., 2003, Aoki et al., 2004, Hwang et al., 2005, Ugawa et al., 2005) indicating the existence of separate populations of dorsal root ganglion neurons with different TRPV1 mRNA and protein staining intensity. In agreement with the findings of a previous qualitative *in situ* hybridization study (Michael and Priestley, 1999), the present quantitative *in situ* hybridization experiments revealed two subpopulations of small and medium-sized neurons that exhibited moderate and high intensities of TRPV1 mRNA expression and TRPV1 immunoreactivity. The two populations of DRG neurons that expressed TRPV1 mRNA or TRPV1 protein could be clearly distinguished through a statistical approach involving ROC analysis based on two characteristic traits of TRPV1-positive neurons: the cell size and the ROD of the mRNA signal or the immunostaining for TRPV1. The quantitative data demonstrated that a distinct subpopulation of small DRG neurons displayed a significantly higher TRPV1 mRNA expression than did a larger

population of small and medium-sized TRPV1-expressing neurons, which accounted for around 19% and 30% of the total neuronal population, respectively, in the L5 DRGs of the rat.

Further, our findings also indicated differences in the regulation and long-lasting alterations in (post-)transcriptional modification of TRPV1 expression following selective chemical and physical injuries inflicted upon primary sensory neurons. In accord with previous reports, peripheral nerve transection resulted in a substantial reduction in the proportion of TRPV1 mRNA-expressing neurons, which was already evident 3 days after surgery and persisted for at least 4 weeks in the L5 DRGs. This was closely paralleled by a significant and persistent decrease in the proportions of TRPV1-immunoreactive neurons in the L5 DRGs. These findings corroborate and extend previous reports of parallel reductions in TRPV1 mRNA expression and protein level in axotomized DRG neurons (Michael and Priestley, 1999). The present study further supported these observations by measurements of TRPV1 mRNA and protein using quantitative RT-PCR and Western blotting, respectively. The results indicated marked, significant and permanent reductions in TRPV1 protein confirming the immunohistochemical analysis. TRPV1 mRNA expression was markedly reduced 3 and 14 days after nerve transection but it showed a moderate increase after 30 days which did not reach significance.

In sharp contrast, following perineural treatment with capsaicin, neurons in the L5 DRG exhibited distinct changes in TRPV1 mRNA and protein expressions. Although the expression of TRPV1 mRNA in type C neurons was markedly decreased 3 days after the treatment, there was a clear-cut tendency toward recovery after 2 weeks, and a statistically significant recovery to about 60% of the control value was evident after a survival period of 4 weeks. In type B neurons, the TRPV1 mRNA expression already displayed a significant reduction by 3 days, with a significant recovery at the end of the study period. The measurements of total TRPV1 mRNA with quantitative RT-PCR in DRGs relating to the capsaicin treated sciatic nerve confirmed these findings. An early profound decrease in TRPV1 mRNA expression was followed by a clear-cut tendency to recovery resulting in a significant increase in TRPV1 mRNA expression to about 60 per cent of the control at the end of the study. Interestingly, however, when the TRPV1 immunoreactivity was investigated, a tendency to recovery was not observed. The proportions of TRPV1-immunoreactive type C and type B DRG neurons decreased to about 12% and 25% of the total control neuronal

population after 3 days and remained at these low levels even after a survival period of 4 weeks. It should be noted that these changes in the proportions of affected TRPV1-mRNA expressing and TRPV1-immunoreactive neurons should be considered in light of the fact that about 20% of the neurons in the L5 DRGs are not affected by the lesions for their axons run in nerves other than the sciatic nerve (Yip et al., 1984, Aldskogius et al., 1988). These immunohistochemical findings were strongly supported by measurements of the TRPV1 protein with Western blotting of the L5 DRGs relating to the capsaicin-treated sciatic nerves. The TRPV1 protein was markedly decreased already 3 days after the capsaicin treatment and remained at that low level amounting about 30 per cent of the control throughout the entire period of the study. The long-lasting, apparently irreversible functional impairments observed after perineural capsaicin treatment, such as the abolition of vanilloid-induced chemogenic pain and neurogenic inflammation, elevated latencies of thermal nociceptive reflexes and reduced thermal hyperalgesia, are in accord with the downregulation of TRPV1 protein in the DRG neurons.

Several factors must be considered in the interpretation of the disparate changes brought about by the two types of nerve injuries, which differ substantially in their nature, i.e. nerve transection and perineural capsaicin treatment. In contrast to neurotmesis, although leading to a selective chemodenervation of nociceptive afferents which express the TRPV1 receptor by a mechanism which involves a slowly progressing dying-back type of degeneration process (Jancsó and Lawson, 1990, Jancsó, 1992), perineural treatment with capsaicin leaves the nerve fibres continuous. The exact nature of this denervation process is still unclear, but it has been demonstrated that, although practically all capsaicin-sensitive C-fibre afferents are functionally inactivated, only about half of the axons of this population undergo degeneration, since the number of unmyelinated axons in capsaicin-treated nerves decreased by only some 30% (Jancsó and Lawson, 1990, Pini et al., 1990, Jancsó, 1992). This may imply that after perineural capsaicin, unlike after nerve transection, the surviving axons may provide some trophic support for the chemically injured neurons, which may be sufficient to promote the transcription, but not the translation of TRPV1 mRNA.

Similar phenomena involving a mismatch of mRNA and protein expressions have been reported depending on the developmental and/or functional state of the DRG neurons. Peripherin mRNA and protein have been shown to be expressed in parallel in developing DRG neurons. However, in mature DRGs, large neurons express peripherin mRNA, but not

the protein. This was attributed to changes in the availabilty of peripherally derived trophic factors such as NGF (Goldstein et al., 1996).

Although the distinct changes in the availability of trophic factors probably best explain, at least in part, the findings of the present study, other mechanisms may also be considered. First, peripheral axotomy of primary sensory neurons may be regarded as a trigger for a cellular stress response supporting the mechanisms for survival. This may involve modulation of gene expression, a very tightly regulated process, which depends both on cellular factors and extracellular stimuli. The cell is able to control the expression pattern of its proteome at almost all levels of the flow of the genetic information, including intracellular transport, translation and the turnover of the individual mRNAs (Eberhardt et al., 2007). Comparative proteomic studies revealed the lack of correlation between the mRNA and the protein levels of numerous genes (Gygi et al., 1999, Celis et al., 2000, Rajasekhar and Holland, 2004), indicating that post-transcriptional regulation is more important, than often assumed. There is a time lag associated with synthesis, processing and export of *de novo* synthesized mRNA, thus the use of existing mRNA by a controlled selective and reversible silencing is more suitable when immediate changes is required (Mazumder et al., 2003, 2010, Sonenberg and Hinnebusch, 2007). This mechanism is more effective in tegulating a biological process rather than to interrupt it later to overcome the intracellular accumulation of proteins (Holcik and Sonenberg, 2005, Sonenberg and Hinnebusch, 2007, 2009).

Another way to regulate the cellular mRNA pool could be to send them temporarily into processing bodies or building up stress granules consisting of untranslated mRNAs (Nover et al., 1989, Bashkirov et al., 1997). Selective recruitment of specific mRNA transcripts into stress granules is thought to regulate their stability and translation (Anderson and Kedersha, 2002). Finally, neuronal granules harbor translationally silenced mRNAs that are transported to nerve cell processes, where they are released and translated in response to specific exogenous stimuli (Krichevsky and Kosik, 2001). Processing bodies, stress granules and neuronal granules are dynamic; mRNAs can be transported between these compartments as a rapid and reversible cellular response to stressful stimuli (Thomas et al., 2005, Anderson and Kedersha, 2006, Parker and Sheth, 2007).

Recent findings showing the replacement of chemically injured neurons by proliferating DRG cells may suggest an alternative possibility for the partial restitution of the neuron populations which express TRPV1 mRNA. Indeed, the results demonstrated a

restoration by neurogenesis of viscerosensory innervation following a systemic injection of capsaicin (Czaja et al., 2008) which results in the degeneration of large populations of nodose and DRG neurons (Jancsó et al., 1977, 1980b, 1985, Ritter and Dinh, 1988, Jancsó and Lawson, 1990, Jancsó, 1992, Hiura et al., 2002, Hiura, 2009). However, this possibility seems unlikely, since little if any functional recovery was demonstrated after perineural treatment with capsaicin (Jancsó et al., 1980a, Fitzgerald, 1983, Jancsó and Lawson, 1990, Jancsó, 1992, Dux et al., 1998, Sántha and Jancsó, 2003, Jancsó et al., 2011).

In conclusion, the observations summarized in this Thesis have revealed new mechanisms in the development of lesion-induced neuroplastic changes of the somatosensory system. The findings disclosed that, in contrast to the widely held view, the changes in the spinal distribution of CTB-binding afferents following peripheral nerve lesions may be accounted for by a phenotypic switch of C-fibre primary afferent fibres rather than a sprouting response of myelinated A-fibre afferents. Peripheral nerve section results in an increased labelling of the substantia gelatinosa of the spinal dorsal horn with CTB due to a phenotypic switch of C-fibre afferents involving an increased expression of the GM1 ganglioside. Since changes in neural gangliosides may affect the NGF-regulated expression of specific proteins of nociceptive primary afferents, the expression of the archetypic nociceptive ion channel, the capsaicin/TRPV1 channel was also investigated.

By making use of *in situ* hybridization and immunohistochemistry and a statistical approach, separate classes of TRPV1-expressing DRG neurons were identified. Further, using *in situ* hybridization, immunohistochemistry, QRT-PCR and Western blotting we found distinct and disparate changes in TRPV1 mRNA and protein expressions following peripheral nerve injuries. Both transection and perineural capsaicin treatment of the sciatic nerve resulted in a dramatic and long-lasting (up to 4 weeks) reduction in the number of TRPV1-immunoreactive neurons. In contrast, *in situ* hybridization and QRT-PCR findings demonstrated a clear cut tendency for recovery toward normal levels of TRPV1 mRNA after perineural capsaicin treatment but not after nerve transection. The reduction in the expression of the TRPV1 receptor protein may explain the highly selective analgesic, anti-hyperalgesic and anti-inflammatory actions of perineurally applied capsaicin.

The present findings may also have important implications as concerns the mechanism(s) of chemically induced selective analgesia. The results point to the possibility that interfering with the translation and/or post-translational processing of nociceptive ion

channels, such as the TRPV1 receptor, by using specific siRNAs, for example, may offer a novel approach to pain relief by employing molecular biological tools.

6. ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisors, Professor Gábor Jancsó, head of the Department of Physiology Faculty of Medicine, for the opportunity to work at his department, for all of his guidance and valuable help both on scientific and human sides and Dr. Péter Sántha, for his help and attitude through the whole period of the work. I am grateful to Professor Károly Gulya, head of the Department of Cell Biology and Molecular Medicine, Faculty of Science and Informatics, for his help and for supporting me to complete my thesis and run my teaching activities at the same time at the two different departments. I would like to thank Dr. Tibor Nyári, for his outstanding help in the statistical interpretation of our results and Dr. Mária Dux for her scientific advices. I am also thankful to Zsuzsa Ambrus and Éva Hegyeshalmi for the excellent technical assistance. I would like to thank all of my colleagues at both departments who helped me during the project. Finally, I would like to thank my family for the support, understanding and patience during these years.

7. REFERENCES

Aguayo LG, White G (1992) Effects of nerve growth factor on TTX- and capsaicin-sensitivity in adult rat sensory neurons. Brain Res 570:61-67.

Albers KM, Woodbury CJ, Ritter AM, Davis BM, Koerber HR (2006) Glial cell-line-derived neurotrophic factor expression in skin alters the mechanical sensitivity of cutaneous nociceptors. JNeurosci 26:2981-2990.

Aldskogius H, Arvidsson J, Grant G (1985) The reaction of primary sensory neurons to peripheral nerve injury with particular emphasis on transganglionic changes. Brain Res 357:27-46.

Aldskogius H, Wiesenfeldhallin Z, Kristensson K (1988) Selective Neuronal Destruction by Ricinus-Communis Agglutinin-I and Its Use for the Quantitative-Determination of Sciatic-Nerve Dorsal-Root Ganglion-Cell Numbers. Brain Research 461:215-220.

Anderson P, Kedersha N (2002) Stressful initiations. J Cell Sci 115:3227-3234.

Anderson P, Kedersha N (2006) RNA granules. J Cell Biol 172:803-808.

Andreev N, Dimitrieva N, Koltzenburg M, McMahon SB (1995) Peripheral administration of nerve growth factor in the adult rat produces a thermal hyperalgesia that requires the presence of sympathetic post-ganglionic neurones. Pain 63:109-115.

Aoki Y, Takahashi Y, Ohtori S, Moriya H, Takahashi K (2004) Distribution and immunocytochemical characterization of dorsal root ganglion neurons innervating the lumbar intervertebral disc in rats: a review. Life Sci 74:2627-2642.

Armitage P, Colton T (2005) Encyclopedia of biostatistics. Chichester, West Sussex, England; Hoboken, NJ: John Wiley.

Armitage P CT (2001) Biostatistics in clinical trial. Chichester: Wiley.

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A (2004) Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 41:849-857.

Bao L, Wang HF, Cai HJ, Tong YG, Jin SX, Lu YJ, Grant G, Hökfelt T, Zhang X (2002) Peripheral axotomy induces only very limited sprouting of coarse myelinated afferents into inner lamina II of rat spinal cord. EurJNeurosci 16:175-185.

Baranowski R, Lynn B, Pini A (1986) The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. BrJPharmacol 89:267-276.

Bashkirov VI, Scherthan H, Solinger JA, Buerstedde JM, Heyer WD (1997) A mouse cytoplasmic exoribonuclease (mXRN1p) with preference for G4 tetraplex substrates. J Cell Biol 136:761-773.

Benham CD, Gunthorpe MJ, Davis JB (2003) TRPV channels as temperature sensors. Cell Calcium 33:479-487.

Bevan S, Winter J (1995) Nerve growth factor (NGF) differentially regulates the chemosensitivity of adult rat cultured sensory neurons. JNeurosci 15:4918-4926.

Bhave G, Zhu W, Wang H, Brasier DJ, Oxford GS, Gereau RWt (2002) cAMP-dependent protein kinase regulates desensitization of the capsaicin receptor (VR1) by direct phosphorylation. Neuron 35:721-731.

Bianchi BR, Lee CH, Jarvis MF, El Kouhen R, Moreland RB, Faltynek CR, Puttfarcken PS (2006) Modulation of human TRPV1 receptor activity by extracellular protons and host cell expression system. Eur J Pharmacol 537:20-30.

Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V (2001) Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol 134:845-852.

Breese NM, George AC, Pauers LE, Stucky CL (2005) Peripheral inflammation selectively increases TRPV1 function in IB4-positive sensory neurons from adult mouse. Pain 115:37-49.

Bridges D, Rice AS, Egertova M, Elphick MR, Winter J, Michael GJ (2003) Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. Neuroscience 119:803-812.

Brown AG (1981) Organization in the spinal cord: the anatomy and physiology of identified neurones. Berlin ; New York: Springer-Verlag.

Calixto JB, Kassuya CA, Andre E, Ferreira J (2005) Contribution of natural products to the discovery of the transient receptor potential (TRP) channels family and their functions. Pharmacol Ther 106:179-208.

Caterina MJ, Julius D (1999) Sense and specificity: a molecular identity for nociceptors. CurrOpinNeurobiol 9:525-530.

Caterina MJ, Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 24:487-517.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306-313.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816-824.

Cavanaugh DJ, Chesler AT, Jackson AC, Sigal YM, Yamanaka H, Grant R, O'Donnell D, Nicoll RA, Shah NM, Julius D, Basbaum AI (2011) Trpv1 reporter mice reveal highly restricted brain distribution and functional expression in arteriolar smooth muscle cells. J Neurosci 31:5067-5077.

Celis JE, Kruhoffer M, Gromova I, Frederiksen C, Ostergaard M, Thykjaer T, Gromov P, Yu J, Palsdottir H, Magnusson N, Orntoft TF (2000) Gene expression profiling: monitoring transcription and translation products using DNA microarrays and proteomics. FEBS Lett 480:2-16.

Cervero F (1994) Sensory innervation of the viscera: peripheral basis of visceral pain. Physiol Rev 74:95-138.

Cervero F (1995) Visceral pain: mechanisms of peripheral and central sensitization. Ann Med 27:235-239.

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D (2001) Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 411:957-962.

Chung JM, Lee KH, Hori Y, Willis WD (1985) Effects of capsaicin applied to a peripheral nerve on the responses of primate spinothalamic tract cells. Brain Res 329:27-38.

Clapham DE (1997) Some like it hot: spicing up ion channels. Nature 389:783-784.

Cortright DN, Krause JE, Broom DC (2007) TRP channels and pain. Biochim Biophys Acta 1772:978-988.

Czaja K, Burns GA, Ritter RC (2008) Capsaicin-induced neuronal death and proliferation of the primary sensory neurons located in the nodose ganglia of adult rats. Neuroscience 154:621-630.

Csillik B, Knyihár-Csillik E (1986) The protein gate: structure and plasticity of the primary nociceptive analyzer. Budapest: Akadémiai Kiadó.

Csillik B, Knyihár E (1978) Biodynamic plasticity in the Rolando substance. ProgNeurobiol 10:203-230.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183-187.

Di Marzo V, Blumberg PM, Szallasi A (2002) Endovanilloid signaling in pain. Curr Opin Neurobiol 12:372-379.

Diamond J, Holmes M, Coughlin M (1992) Endogenous NGF and nerve impulses regulate the collateral sprouting of sensory axons in the skin of the adult rat. J Neurosci 12:1454-1466.

Dmitrieva N, McMahon SB (1996) Sensitisation of visceral afferents by nerve growth factor in the adult rat. Pain 66:87-97.

Docherty RJ, Yeats JC, Bevan S, Boddeke HW (1996) Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. Pflugers Arch 431:828-837.

Domoki F, Sántha P, Bari F, Jancsó G (2003) Perineural capsaicin treatment attenuates reactive hyperaemia in the rat skin. NeurosciLett 341:127-130.

Donaldson LF, McQueen DS, Seckl JR (1995) Neuropeptide gene expression and capsaicin-sensitive primary afferents: maintenance and spread of adjuvant arthritis in the rat. JPhysiol 486:473-482.

Dumoulin FL, Raivich G, Streit WJ, Kreutzberg GW (1991) Differential Regulation of Calcitonin Gene-related Peptide (CGRP) in Regenerating Rat Facial Nucleus and Dorsal Root Ganglion. Eur J Neurosci 3:338-342.

Dux M, Sann H, Jancsó G (1998) Changes in fibre populations of the rat hairy skin after selective chemodenervation by capsaicin. European Journal of Neuroscience 10:299-299.

Eberhardt W, Doller A, Akool el S, Pfeilschifter J (2007) Modulation of mRNA stability as a novel therapeutic approach. Pharmacol Ther 114:56-73.

Fitzgerald M (1983) Capsaicin and sensory neurones--a review. Pain 15:109-130.

Fitzgerald M, Woolf CJ (1982) The time course and specificity of the changes in the behavioural and dorsal horn cell responses to noxious stimuli following peripheral nerve capsaicin treatment in the rat. Neuroscience 7:2051-2056.

Flockerzi V (2007) An introduction on TRP channels. Handb Exp Pharmacol 1-19.

Gamse R, Holzer P, Lembeck F (1980) Decrease of substance P in primary afferent neurones and impairment of neurogenic plasma extravasation by capsaicin. BrJPharmacol 68:207-213.

Gamse R, Leeman SE, Holzer P, Lembeck F (1981) Differential effects of capsaicin on the content of somatostatin, substance P, and neurotensin in the nervous system of the rat. Naunyn Schmiedebergs ArchPharmacol 317:140-148.

Gamse R, Petsche U, Lembeck F, Jancsó G (1982) Capsaicin Applied to Peripheral-Nerve Inhibits Axoplasmic-Transport of Substance-P and Somatostatin. Brain Research 239:447-462.

Gibson SJ, McGregor G, Bloom SR, Polak JM, Wall PD (1982) Local application of capsaicin to one sciatic nerve of the adult rat induces a marked depletion in the peptide content of the lumbar dorsal horn. Neuroscience 7:3153-3162.

Goldstein ME, Grant P, House SB, Henken DB, Gainer H (1996) Developmental regulation of two distinct neuronal phenotypes in rat dorsal root ganglia. Neuroscience 71:243-258.

Grant G, Arvidsson J (1975) Transganglionic degeneration in trigeminal primary sensory neurons. Brain Res 95:265-279.

Grant G, Ygge J (1981) Somatotopic organization of the thoracic spinal nerve in the dorsal horn demonstrated with transganglionic degeneration. J Comp Neurol 202:357-364.

Green EC (1968) Anatomy of the rat. New York: Hafner.

Guo A, Vulchanova L, Wang J, Li X, Elde R (1999) Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X3 purinoceptor and IB4 binding sites. Eur J Neurosci 11:946-958.

Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 19:1720-1730.

Hall ME (1982) Changes in synthesis of specific proteins in axotomized dorsal root ganglia. Exp Neurol 76:83-93.

Helliwell RJ, McLatchie LM, Clarke M, Winter J, Bevan S, McIntyre P (1998) Capsaicin sensitivity is associated with the expression of the vanilloid (capsaicin) receptor (VR1) mRNA in adult rat sensory ganglia. Neurosci Lett 250:177-180.

Hiura A (2000) Neuroanatomical effects of capsaicin on the primary afferent neurons. ArchHistolCytol 63:199-215.

Hiura A (2009) Is thermal nociception only sensed by the capsaicin receptor, TRPV1? AnatSciInt 84:122-128.

Hiura A, Nakae Y, Nakagawa H (2002) Cell death of primary afferent nerve cells in neonatal mice treated with capsaicin. AnatSciInt 77:47-50.

Hökfelt T (1991) Neuropeptides in perspective: the last ten years. Neuron 7:867-879.

Hökfelt T, Kellerth JO, Nilsson G, Pernow B (1975) Substance p: localization in the central nervous system and in some primary sensory neurons. Science 190:889-890.

Hökfelt T, Wiesenfeld-Hallin Z, Villar M, Melander T (1987) Increase of galanin-like immunoreactivity in rat dorsal root ganglion cells after peripheral axotomy. Neurosci Lett 83:217-220.

Hökfelt T, Zhang X, Wiesenfeld-Hallin Z (1994) Messenger plasticity in primary sensory neurons following axotomy and its functional implications. Trends Neurosci 17:22-30.

Holcik M, Sonenberg N (2005) Translational control in stress and apoptosis. Nat Rev Mol Cell Biol 6:318-327.

Holzer P (1988) Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. Neuroscience 24:739-768.

Holzer P (1991) Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. PharmacolRev 43:143-201.

Holzer P (1998) Neurogenic vasodilatation and plasma leakage in the skin. GenPharmacol 30:5-11.

Holzer P (2008) The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nocisensor. BrJPharmacol 155:1145-1162.

Hwang SJ, Oh JM, Valtschanoff JG (2005) Expression of the vanilloid receptor TRPV1 in rat dorsal root ganglion neurons supports different roles of the receptor in visceral and cutaneous afferents. Brain Res 1047:261-266.

Immke DC, Gavva NR (2006) The TRPV1 receptor and nociception. Semin Cell Dev Biol 17:582-591.

Jancsó G (1981) Intracisternal capsaicin: selective degeneration of chemosensitive primary sensory afferents in the adult rat. Neurosci Lett 27:41-45.

Jancsó G (1992) Pathobiological reactions of C-fibre primary sensory neurones to peripheral nerve injury. ExpPhysiol 77:405-431.

Jancsó G (2009) Neurogenic Inflammation in Health and Disease. Amsterdam: Elsevier.

Jancsó G, Ambrus A (1994) Capsaicin sensitivity of primary sensory neurones and its regulation. In: Peripheral neurons in nociception: physio-pharmacological aspects, vol. 1 (Besson, J. M. et al., eds), pp 71-87 Paris: John Libbey Eurotext.

Jancsó G, Chahl J, Szolcsányi J, Lembeck F (1984) Sensory nerves as modulators of inflammatory reactions. In: Antidromic Vasodilatation and Neurogenic Inflammation, pp 207-222 Budapest: Akadémiai Kiadó.

Jancsó G, Dux M, Oszlács O, Sántha P (2008) Activation of the transient receptor potential vanilloid-1 (TRPV1) channel opens the gate for pain relief. British Journal of Pharmacology 155:1139-1141.

Jancsó G, Jancsó-Gábor A (1980) Effect of Capsaicin on Morphine Analgesia - Possible Involvement of Hypothalamic Structures. Naunyn-Schmiedebergs Archives of Pharmacology 311:285-288.

Jancsó G, Király E (1980) Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. JComp Neurol 190:781-792.

Jancsó G, Király E (1981) Sensory neurotoxins: chemically induced selective destruction of primary sensory neurons. Brain Res 210:83-89.

Jancsó G, Király E, Jancsó-Gábor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270:741-743.

Jancsó G, Király E, Jancsó-Gábor A (1980a) Chemosensitive pain fibres and inflammation. IntJTissue React 2:57-66.

Jancsó G, Király E, Jancsó-Gábor A (1980b) Direct evidence for an axonal site of action of capsaicin. Naunyn Schmiedebergs Arch Pharmacol 313:91-94.

Jancsó G, Király E, Joó F, Such G, Nagy A (1985) Selective Degeneration by Capsaicin of A Subpopulation of Primary Sensory Neurons in the Adult-Rat. Neuroscience Letters 59:209-214.

Jancsó G, Király E, Such G, Joó F, Nagy A (1987) Neurotoxic effect of capsaicin in mammals. Acta Physiol Hung 69:295-313.

Jancsó G, Knyihár E (1975) Functional linkage between nociception and fluoride-resistant acid phosphatase activity in the Rolando substance. Neurobiology 5:42-43.

Jancsó G, Lawson SN (1987) Perineural Capsaicin Treatment of the Sciatic-Nerve in Adult-Rats Causes Transganglionic Changes in the Spinal-Cord Dorsal Horn. Journal of Physiology-London 394:109-109.

Jancsó G, Lawson SN (1988) Ganglionic Changes Associated with Transganglionic Degeneration of Capsaicin-Sensitive Primary Sensory Afferents - A Quantitative Morphometric and Immunohistochemical Study. Regulatory Peptides 22:97-97.

Jancsó G, Lawson SN (1990) Transganglionic degeneration of capsaicin-sensitive C-fiber primary afferent terminals. Neuroscience 39:501-511.

Jancsó G, Oszlács O, Sántha P (2011) The capsaicin paradox: pain relief by an algesic agent. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry 10:52-56.

Jancsó G, Sántha P, Gecse K (2002) Peripheral nerve lesion-induced uptake and transport of choleragenoid by capsaicin-sensitive C-fibre spinal ganglion neurons. Acta Biologica Hungarica 53:77-84.

Jancsó G, Sántha P, Szigeti C, Dux M (2004) Selective C-fiber deafferentation of the spinal dorsal horn prevents lesion-induced transganglionic transport of choleragenoid to the substantia gelatinosa in the rat. Neurosci Lett 361:204-207.

Jancsó G, Such G (1983) Effects of Capsaicin Applied Perineurally to the Vagus Nerve on Cardiovascular and Respiratory Functions in the Cat. Journal of Physiology-London 341:359-370.

Jancsó G, Such G, Rödel C, Sicuteri F, Vecchiet L, Fanciullacci M (1987) A new approach to selective regional analgesia. In: Trends in cluster headache, pp 59 -68 Amsterdam, New York: Excerpta Medica.

Jancsó N (1960) Role of the nerve terminals in the mechanism of inflammatory reactions. BullMillard Fillmore Hosp 7 53-77.

Jancsó N (1968) Desensitization with capsaicin as a tool for studying the function of pain receptors In: Pharmacology of pain(Lim, R. K. S., ed), pp 33-55 Oxford: Pergamon Press.

Jancsó N, Jancsó-Gábor A, Szolcsányi J (1967) Direct evidence for neurogenic inflammation and its prevention by denervation and by pretreatment with capsaicin. Br J Pharmacol Chemother 31:138-151.

Jancsó N, Jancsó-Gábor A, Szolcsányi J (1968) The role of sensory nerve endings in neurogenic inflammation induced in human skin and in the eye and paw of the rat. BrJPharmacolChemother 33:32-41.

Jeske NA, Patwardhan AM, Ruparel NB, Akopian AN, Shapiro MS, Henry MA (2009) A-kinase anchoring protein 150 controls protein kinase C-mediated phosphorylation and sensitization of TRPV1. Pain 146:301-307.

Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. Nature 413:203-210.

Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U (2004) Phosphorylation of vanilloid receptor 1 by Ca2+/calmodulin-dependent kinase II regulates its vanilloid binding. J Biol Chem 279:7048-7054.

Kasama S, Kawakubo M, Suzuki T, Nishizawa T, Ishida A, Nakayama J (2007) RNA interference-mediated knock-down of transient receptor potential vanilloid 1 prevents forepaw inflammatory hyperalgesia in rat. EurJNeurosci 25:2956-2963.

Kissin I, Bright CA, Bradley EL, Jr. (2002) Selective and long-lasting neural blockade with resiniferatoxin prevents inflammatory pain hypersensitivity. AnesthAnalg 94:1253-1258.

Kissin I, Freitas CF, Bradley EL, Jr. (2007) Perineural resiniferatoxin prevents the development of hyperalgesia produced by loose ligation of the sciatic nerve in rats. AnesthAnalg 104:1210-1216.

Knotkova H, Pappagallo M, Szallasi A (2008) Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival? ClinJPain 24:142-154.

Kohama I, Ishikawa K, Kocsis JD (2000) Synaptic reorganization in the substantia gelatinosa after peripheral nerve neuroma formation: aberrant innervation of lamina II neurons by Abeta afferents. J Neurosci 20:1538-1549.

Krichevsky AM, Kosik KS (2001) Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. Neuron 32:683-696.

Lee H, Caterina MJ (2005) TRPV channels as thermosensory receptors in epithelial cells. Pflugers Arch 451:160-167.

Lee SY, Lee JH, Kang KK, Hwang SY, Choi KD, Oh U (2005) Sensitization of vanilloid receptor involves an increase in the phosphorylated form of the channel. Arch Pharm Res 28:405-412.

Lekan HA, Carlton SM, Coggeshall RE (1996) Sprouting of A beta fibers into lamina II of the rat dorsal horn in peripheral neuropathy. Neurosci Lett 208:147-150.

Leon A, Benvegnu D, Dal Toso R, Presti D, Facci L, Giorgi O, Toffano G (1984) Dorsal root ganglia and nerve growth factor: a model for understanding the mechanism of GM1 effects on neuronal repair. JNeurosciRes 12:277-287.

Levine JD, Alessandri-Haber N (2007) TRP channels: targets for the relief of pain. Biochim Biophys Acta 1772:989-1003.

Lewin GR, Ritter AM, Mendell LM (1993) Nerve growth factor-induced hyperalgesia in the neonatal and adult rat. J Neurosci 13:2136-2148.

Lieberman AR (1971) The axon reaction: a review of the principal features of perikaryal responses to axon injury. Int Rev Neurobiol 14:49-124.

Lindsay RM, Harmar AJ (1989) Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. Nature 337:362-364.

Liu L, Simon SA (1996) Similarities and differences in the currents activated by capsaicin, piperine, and zingerone in rat trigeminal ganglion cells. J Neurophysiol 76:1858-1869.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Maggi CA, Meli A (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. GenPharmacol 19:1-43.

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor, N.Y.: Cold Spring Harbor Laboratory.

Mazumder B, Li X, Barik S (2010) Translation control: a multifaceted regulator of inflammatory response. J Immunol 184:3311-3319.

Mazumder B, Seshadri V, Fox PL (2003) Translational control by the 3'-UTR: the ends specify the means. Trends Biochem Sci 28:91-98.

McMahon SB, Dmitrieva N, Koltzenburg M (1995) Visceral pain. Br J Anaesth 75:132-144.

McMahon SB, Gibson S (1987) Peptide expression is altered when afferent nerves reinnervate inappropriate tissue. Neurosci Lett 73:9-15.

Mesulam MM (1978) Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents. J Histochem Cytochem 26:106-117.

Mezey E, Toth ZE, Cortright DN, Arzubi MK, Krause JE, Elde R, Guo A, Blumberg PM, Szallasi A (2000) Distribution of mRNA for vanilloid receptor subtype 1 (VR1), and VR1-like immunoreactivity, in the central nervous system of the rat and human. Proc Natl Acad Sci U S A 97:3655-3660.

Michael GJ, Priestley JV (1999) Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its downregulation by axotomy. J Neurosci 19:1844-1854.

Millan MJ (1999) The induction of pain: an integrative review. Prog Neurobiol 57:1-164.

Miller MS, Buck SH, Sipes IG, Yamamura HI, Burks TF (1982) Regulation of substance P by nerve growth factor: disruption by capsaicin. Brain Res 250:193-196.

Minke B (1977) Drosophila mutant with a transducer defect. Biophys Struct Mech 3:59-64.

Minke B, Parnas M (2006) Insights on TRP channels from in vivo studies in Drosophila. Annu Rev Physiol 68:649-684.

Mitchell V, Feyereisen K, Bouret S, Leroy D, Beauvillain JC (2001) Microwave strategy for improving the simultaneous detection of estrogen receptor and galanin receptor mRNA in the rat hypothalamus. J Histochem Cytochem 49:901-910.

Montell C, Rubin GM (1989) Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. Neuron 2:1313-1323.

Mutoh T, Tokuda A, Inokuchi J, Kuriyama M (1998) Glucosylceramide synthase inhibitor inhibits the action of nerve growth factor in PC12 cells. JBiolChem 273:26001-26007.

Nagy I, Sántha P, Jancsó G, Urban L (2004) The role of the vanilloid (capsaicin) receptor (TRPV1) in physiology and pathology. Eur J Pharmacol 500:351-369.

Nagy JI, Hunt SP (1983) The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment. JComp Neurol 218:145-158.

Nagy JI, Iversen LL, Goedert M, Chapman D, Hunt SP (1983) Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat. JNeurosci 3:399-406.

Nakamura S, Myers RR (1999) Myelinated afferents sprout into lamina II of L3-5 dorsal horn following chronic constriction nerve injury in rats. Brain Res 818:285-290.

Nelson EK (1919) The constitution of capsaicin - the pungent principle of capsicum. J Am Chem Soc 41:1115-1119.

Nielsch U, Bisby MA, Keen P (1987) Effect of cutting or crushing the rat sciatic nerve on synthesis of substance P by isolated L5 dorsal root ganglia. Neuropeptides 10:137-145.

Nilius B, Mahieu F, Karashima Y, Voets T (2007) Regulation of TRP channels: a voltage-lipid connection. BiochemSocTrans 35:105-108.

Nilius B, Voets T (2004) Diversity of TRP channel activation. Novartis Found Symp 258:140-149; discussion 149-159, 263-146.

Noguchi K, Senba E, Morita Y, Sato M, Tohyama M (1990) Alpha-CGRP and beta-CGRP mRNAs are differentially regulated in the rat spinal cord and dorsal root ganglion. Brain Res Mol Brain Res 7:299-304.

Nolano M, Simone DA, Wendelschafer-Crabb G, Johnson T, Hazen E, Kennedy WR (1999) Topical capsaicin in humans: parallel loss of epidermal nerve fibers and pain sensation. Pain 81:135-145.

Nover L, Scharf KD, Neumann D (1989) Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol Cell Biol 9:1298-1308.

Oszlács O, Sántha P, Jancsó G (2009) Long-Lasting Antinocicepttve and Anti-Inflammatory Effects of N-Oleoyldopamine, An Endogenous Vanilloid. Neuropeptides 43:413-413.

Palermo NN, Brown HK, Smith DL (1981) Selective neurotoxic action of capsaicin on glomerular C-type terminals in rat substantia gelatinosa. Brain Res 208:506-510.

Pare M, Elde R, Mazurkiewicz JE, Smith AM, Rice FL (2001) The Meissner corpuscle revised: a multiafferented mechanoreceptor with nociceptor immunochemical properties. J Neurosci 21:7236-7246.

Parker R, Sheth U (2007) P bodies and the control of mRNA translation and degradation. Mol Cell 25:635-646.

Pedersen SF, Owsianik G, Nilius B (2005) TRP channels: an overview. Cell Calcium 38:233-252.

Pertens E, Urschel-Gysbers BA, Holmes M, Pal R, Foerster A, Kril Y, Diamond J (1999) Intraspinal and behavioral consequences of nerve growth factor-induced nociceptive sprouting and nerve growth factor-induced hyperalgesia compared in adult rats. J Comp Neurol 410:73-89.

Petho G, Izydorczyk W, Reeh PW (2004) Effects of TRPV1 receptor antagonists on stimulated iCGRP release from isolated skin of rats and TRPV1 mutant mice. Pain 109:284-290.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29:e45.

Pini A, Baranowski R, Lynn B (1990) Long-Term Reduction in the Number of C-Fibre Nociceptors Following Capsaicin Treatment of a Cutaneous Nerve in Adult Rats. EurJNeurosci 2:89-97.

Pórszász J, Jancsó N (1959) Studies on the action potentials of sensory nerves in animals desensitized with capsaicine. Acta Physiol Acad Sci Hung 16:299-306.

Pospisilova E, Palecek J (2006) Post-operative pain behavior in rats is reduced after single high-concentration capsaicin application. Pain 125:233-243.

Rajasekhar VK, Holland EC (2004) Postgenomic global analysis of translational control induced by oncogenic signaling. Oncogene 23:3248-3264.

Rangell LK, Keller GA (2000) Application of microwave technology to the processing and immunolabeling of plastic-embedded and cryosections. J Histochem Cytochem 48:1153-1159.

Reilly DM, Ferdinando D, Johnston C, Shaw C, Buchanan KD, Green MR (1997) The epidermal nerve fibre network: characterization of nerve fibres in human skin by confocal microscopy and assessment of racial variations. BrJDermatol 137:163-170.

Relf BL, Machaalani R, Waters KA (2002) Retrieval of mRNA from paraffin-embedded human infant brain tissue for non-radioactive in situ hybridization using oligonucleotides. J Neurosci Methods 115:129-136.

Réthelyi M, Salim MZ, Jancsó G (1986) Altered Distribution of Dorsal-Root Fibers in the Rat Following Neonatal Capsaicin Treatment. Neuroscience 18:749-761.

Ritter S, Dinh TT (1988) Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. JComp Neurol 271:79-90.

Robertson B, Grant G (1985) A comparison between wheat germ agglutinin-and choleragenoid-horseradish peroxidase as anterogradely transported markers in central branches of primary sensory neurones in the rat with some observations in the cat. Neuroscience 14:895-905.

Robertson B, Grant G (1989) Immunocytochemical evidence for the localization of the GM1 ganglioside in carbonic anhydrase-containing and RT 97-immunoreactive rat primary sensory neurons. JNeurocytol 18:77-86.

Sann H, Pierau FK (1998) Efferent functions of C-fiber nociceptors. Z Rheumatol 57 Suppl 2:8-13.

Sántha P, Jancsó G (2003) Transganglionic transport of choleragenoid by capsaicin-sensitive C-fibre afferents to the substantia gelatinosa of the spinal dorsal horn after peripheral nerve section. Neuroscience 116:621-627.

Schmidt R, Schmelz M, Forster C, Ringkamp M, Torebjork E, Handwerker H (1995) Novel classes of responsive and unresponsive C nociceptors in human skin. J Neurosci 15:333-341.

Schwartz M, Spirman N (1982) Sprouting from chicken embryo dorsal root ganglia induced by nerve growth factor is specifically inhibited by affinity-purified antiganglioside antibodies. ProcNatlAcadSciUSA 79:6080-6083.

Seddon HJ (1943) Three types of nerve injury. Brain 66:237-246.

Shehab SA, Atkinson ME (1986) Vasoactive intestinal polypeptide (VIP) increases in the spinal cord after peripheral axotomy of the sciatic nerve originate from primary afferent neurons. Brain Res 372:37-44.

Shehab SA, Spike RC, Todd AJ (2003) Evidence against cholera toxin B subunit as a reliable tracer for sprouting of primary afferents following peripheral nerve injury. Brain Res 964:218-227.

Shi TJ, Tandrup T, Bergman E, Xu ZQ, Ulfhake B, Hökfelt T (2001) Effect of peripheral nerve injury on dorsal root ganglion neurons in the C57 BL/6J mouse: marked changes both in cell numbers and neuropeptide expression. Neuroscience 105:249-263.

Sonenberg N, Hinnebusch AG (2007) New modes of translational control in development, behavior, and disease. Mol Cell 28:721-729.

Sonenberg N, Hinnebusch AG (2009) Regulation of translation initiation in eukaryotes: mechanisms and biological targets. Cell 136:731-745.

Starowicz K, Nigam S, Di Marzo V (2007) Biochemistry and pharmacology of endovanilloids. Pharmacol Ther 114:13-33.

Szallasi A, Blumberg PM (1990) Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. Brain Res 524:106-111.

Szallasi A, Blumberg PM (1999) Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol Rev 51:159-212.

Szallasi A, Blumberg PM, Nilsson S, Hökfelt T, Lundberg JM (1994) Visualization by [3H]resiniferatoxin autoradiography of capsaicin-sensitive neurons in the rat, pig and man. Eur J Pharmacol 264:217-221.

Szentágothai J (1964) Neuronal and Synaptic Arrangement in the Substantia Gelatinosa Rolandi. J Comp Neurol 122:219-239.

Szigeti C, Kovács B, Körtvély E, Gulya K (2003) Comparison of treatment regimens to sensitize in situ hybridization for low-abundance calmodulin transcripts in the white matter of the rat spinal cord. Acta Biologica Szegediensis 47:1-6.

Tetzlaff W, Kreutzberg GW (1985) Ornithine decarboxylase in motoneurons during regeneration. Exp Neurol 89:679-688.

Thomas MG, Martinez Tosar LJ, Loschi M, Pasquini JM, Correale J, Kindler S, Boccaccio GL (2005) Staufen recruitment into stress granules does not affect early mRNA transport in oligodendrocytes. Mol Biol Cell 16:405-420.

Tognetto M, Amadesi S, Harrison S, Creminon C, Trevisani M, Carreras M, Matera M, Geppetti P, Bianchi A (2001) Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. J Neurosci 21:1104-1109.

Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M, Kuraishi Y (2001) Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. J Neurochem 76:1628-1635.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531-543.

Tominaga M, Tominaga T (2005) Structure and function of TRPV1. Pflugers Arch 451:143-150.

Tong YG, Wang HF, Ju G, Grant G, Hökfelt T, Zhang X (1999) Increased uptake and transport of cholera toxin B-subunit in dorsal root ganglion neurons after peripheral axotomy: possible implications for sensory sprouting. JComp Neurol 404:143-158.

Toth-Kása I, Jancsó G, Obal F, Jr., Husz S, Simon N (1983) Involvement of sensory nerve endings in cold and heat urticaria. J Invest Dermatol 80:34-36.

Toth A, Boczan J, Kedei N, Lizanecz E, Bagi Z, Papp Z, Édes I, Csiba L, Blumberg PM (2005) Expression and distribution of vanilloid receptor 1 (TRPV1) in the adult rat brain. Brain Res Mol Brain Res 135:162-168.

Ugawa S, Ueda T, Yamamura H, Shimada S (2005) In situ hybridization evidence for the coexistence of ASIC and TRPV1 within rat single sensory neurons. Brain Res Mol Brain Res 136:125-133.

Vennekens R, Voets T, Bindels RJ, Droogmans G, Nilius B (2002) Current understanding of mammalian TRP homologues. Cell Calcium 31:253-264.

Verge VM, Wiesenfeld-Hallin Z, Hökfelt T (1993) Cholecystokinin in mammalian primary sensory neurons and spinal cord: in situ hybridization studies in rat and monkey. Eur J Neurosci 5:240-250.

Verge VM, Xu Z, Xu XJ, Wiesenfeld-Hallin Z, Hökfelt T (1992) Marked increase in nitric oxide synthase mRNA in rat dorsal root ganglia after peripheral axotomy: in situ hybridization and functional studies. Proc Natl Acad Sci U S A 89:11617-11621.

Wakisaka S, Kajander KC, Bennett GJ (1991) Increased neuropeptide Y (NPY)-like immunoreactivity in rat sensory neurons following peripheral axotomy. Neurosci Lett 124:200-203.

Wall PD, Fitzgerald M (1982) If substance P fails to fulfil the criteria as a neurotransmitter in somatosensory afferents, what might be its function? Ciba Found Symp 249-266.

Wall PD, Fitzgerald M, Gibson SJ (1981) The response of rat spinal cord cells to unmyelinated afferents after peripheral nerve section and after changes in substance P levels. Neuroscience 6:2205-2215.

Ward SM, Bayguinov J, Won KJ, Grundy D, Berthoud HR (2003) Distribution of the vanilloid receptor (VR1) in the gastrointestinal tract. J Comp Neurol 465:121-135.

Welk E, Petsche U, Fleischer E, Handwerker HO (1983) Altered excitability of afferent C-fibres of the rat distal to a nerve site exposed to capsaicin. NeurosciLett 38:245-250.

White DM (2000) Neurotrophin-3 antisense oligonucleotide attenuates nerve injury-induced Abeta-fibre sprouting. Brain Res 885:79-86.

Wiesenfeld-Hallin Z, Xu XJ, Hakanson R, Feng DM, Folkers K (1990) Plasticity of the peptidergic mediation of spinal reflex facilitation after peripheral nerve section in the rat. Neurosci Lett 116:293-298.

Willis WD, Coggeshall RE (1991) Sensory mechanisms of the spinal cord. New York: Plenum Press.

Winter J, Forbes CA, Sternberg J, Lindsay RM (1988) Nerve growth factor (NGF) regulates adult rat cultured dorsal root ganglion neuron responses to the excitotoxin capsaicin. Neuron 1:973-981.

Winter J, Walpole CS, Bevan S, James IF (1993) Characterization of resiniferatoxin binding sites on sensory neurons: co-regulation of resiniferatoxin binding and capsaicin sensitivity in adult rat dorsal root ganglia. Neuroscience 57:747-757.

Witte DG, Cassar SC, Masters JN, Esbenshade T, Hancock AA (2002) Use of a fluorescent imaging plate reader--based calcium assay to assess pharmacological differences between the human and rat vanilloid receptor. J Biomol Screen 7:466-475.

Wood JN, Docherty R (1997) Chemical activators of sensory neurons. Annu Rev Physiol 59:457-482.

Woolf CJ, Shortland P, Coggeshall RE (1992) Peripheral nerve injury triggers central sprouting of myelinated afferents. Nature 355:75-78.

Woolf CJ, Shortland P, Reynolds M, Ridings J, Doubell T, Coggeshall RE (1995) Reorganization of central terminals of myelinated primary afferents in the rat dorsal horn following peripheral axotomy. JComp Neurol 360:121-134.

Wu ZZ, Chen SR, Pan HL (2005) Transient receptor potential vanilloid type 1 activation down-regulates voltage-gated calcium channels through calcium-dependent calcineurin in sensory neurons. J Biol Chem 280:18142-18151.

Xu XJ, Wiesenfeld-Hallin Z, Villar MJ, Fahrenkrug J, Hökfelt T (1990) On the Role of Galanin, Substance P and Other Neuropeptides in Primary Sensory Neurons of the Rat: Studies on Spinal Reflex Excitability and Peripheral Axotomy. Eur J Neurosci 2:733-743.

Xue Q, Jong B, Chen T, Schumacher MA (2007) Transcription of rat TRPV1 utilizes a dual promoter system that is positively regulated by nerve growth factor. J Neurochem 101:212-222.

Yaksh TL, Farb DH, Leeman SE, Jessell TM (1979) Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. Science 206:481-483.

Yang H, Wanner IB, Roper SD, Chaudhari N (1999) An optimized method for in situ hybridization with signal amplification that allows the detection of rare mRNAs. J Histochem Cytochem 47:431-446.

Yip HK, Rich KM, Lampe PA, Johnson EM (1984) The Effects of Nerve Growth-Factor and Its Antiserum on the Postnatal-Development and Survival After Injury of Sensory Neurons in Rat Dorsal-Root Ganglia. Journal of Neuroscience 4:2986-2992.

Zhang X, Huang J, McNaughton PA (2005) NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. Embo J 24:4211-4223.

Zhang X, Verge V, Wiesenfeld-Hallin Z, Ju G, Bredt D, Synder SH, Hökfelt T (1993) Nitric oxide synthase-like immunoreactivity in lumbar dorsal root ganglia and spinal cord of rat and monkey and effect of peripheral axotomy. J Comp Neurol 335:563-575.

Zhang X, Wiesenfeld-Hallin Z, Hökfelt T (1994) Effect of peripheral axotomy on expression of neuropeptide Y receptor mRNA in rat lumbar dorsal root ganglia. Eur J Neurosci 6:43-57.

Zhu W, Galoyan SM, Petruska JC, Oxford GS, Mendell LM (2004) A developmental switch in acute sensitization of small dorsal root ganglion (DRG) neurons to capsaicin or noxious heating by NGF. J Neurophysiol 92:3148-3152.

APPENDIX

PAPER I.

ELSEVIER

Neuroscience Letters 361 (2004) 204–207

Neuroscience Letters

www.elsevier.com/locate/neulet

Selective C-fiber deafferentation of the spinal dorsal horn prevents lesion-induced transganglionic transport of choleragenoid to the substantia gelatinosa in the rat☆

Gábor Jancsó[a,*], Péter Sántha[a], Csaba Szigeti[a,b], Mária Dux[a]

[a]*Department of Physiology, University of Szeged, Dóm tér 10, H-6720 Szeged, Hungary*
[b]*Department of Zoology and Cell Biology, University of Szeged, Egyetem u. 2, H-6722 Szeged, Hungary*

Abstract

The effect of neonatal capsaicin treatment, producing selective elimination of almost all unmyelinated C-fiber sensory axons, was studied on lesion-induced transganglionic labelling of the substantia gelatinosa of the spinal cord by choleragenoid. In both control and capsaicin-pretreated rats, the injection of choleragenoid-horseradish peroxidase conjugate into the intact sciatic nerves resulted in intense labelling only of the deeper layers of the spinal dorsal horn. In the control but not the capsaicin-pretreated rats, the injection of the tracer into sciatic nerves transected 2 weeks previously produced an intense homogeneous labelling of the substantia gelatinosa. It is concluded that the uptake and axonal transport of choleragenoid by capsaicin-sensitive C-fiber afferents may be accounted for by the lesion-induced transganglionic labelling of the substantia gelatinosa, rather than by A-fiber sprouting.

Keywords: Choleratoxin B subunit; Capsaicin; Sprouting; Pain; Nerve transection; Plasticity; Sensory ganglion

The injection of choleragenoid, the B subunit of choleratoxin (CTB), or its conjugates into chronically injured, but not into intact nerves results in an intense transganglionic labelling of the substantia gelatinosa of the spinal dorsal horn. Choleragenoid has been regarded as a specific marker for myelinated primary afferent fibers which do not normally terminate in the substantia gelatinosa. Accordingly, these findings were interpreted in terms of a sprouting response of myelinated, A-fiber primary afferents entering the substantia gelatinosa ventrally [19,20]. Invasion of the substantia gelatinosa by mechanoreceptive myelinated afferents has been suggested to contribute significantly to neuropathic pain developing after peripheral nerve lesions. This view has been widely accepted, and the presumed sprouting of myelinated primary afferents has been demonstrated in a number of experimental settings involving different types of nerve injuries [7,9,12,13,19,20]. However, recent findings have cast doubt on the sprouting hypothesis of injured myelinated afferent fibers. For instance, an analysis of the size-frequency distribution histograms of choleragenoid-labelled sensory ganglion cells has revealed a substantial increase in the proportion of small neurons after peripheral nerve transection [6,18]. Most of these small neurons belong in the capsaicin-sensitive small cell population of dorsal root ganglion neurons [6,16]. Immunohistochemical studies have demonstrated the co-localization of specific markers of injured unmyelinated primary afferents, e.g. vasoactive intestinal polypeptide and galanin, with choleragenoid in small sensory ganglion neurons and their central terminations [1,17]. Studies making use of the selective neurotoxic effect of capsaicin on the C-fiber primary sensory neurons have indicated that unmyelinated primary afferents may play a significant role in the mechanism of lesion-induced choleragenoid labelling of the substantia gelatinosa [5,6]. More importantly, electron microscopic histochemical studies have demonstrated that a large population of unmyelinated dorsal root axons relating to an injured, but not an intact peripheral nerve transport choleragenoid [16]. These findings afforded direct evidence for the notion that choleragenoid labelling of the substantia gelatinosa after nerve injury may be accounted for by an uptake and transganglionic transport of choleragenoid by the C-fiber afferents rather than by A-fiber sprouting. The present study was initiated in an attempt to furnish further evidence of the critical role of capsaicin-sensitive primary afferents in the mechanism of this phenomenon. An experimental approach was utilized that resulted in a

☆ Dedicated to Professor Manfred Zimmermann on the occassion of his 70th birthday.

* Corresponding author. Tel.: +36-62-545-099/544-577; fax: +36-62-545-842.
E-mail address: jancso@phys.szote.u-szeged.hu (G. Jancsó).

0304-3940/03/$ - see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2003.12.063

selective and permanent elimination of these particular afferent fibers by neonatal treatment with the sensory neurotoxin capsaicin [3,4].

The experiments were performed on adult male Wistar rats weighing 250–300 g. In one group, the rats ($n = 4$) were pretreated with a single dose of capsaicin (50 mg/kg, Fluka, Switzerland), administered subcutaneously under ether anaesthesia on the second day of life. This treatment is known to result in the selective degeneration of small B-type primary sensory neurons which give rise to unmyelinated axons [3,4]. In another group, four animals injected with the solvent for capsaicin (8% ethanol, 6% Tween 80 in saline) served as controls.

Three months later, the animals were anaesthetized with chloral hydrate (400 mg/kg, i.p., Reanal, Hungary,) and the right sciatic nerve was exposed in the midthigh and transected distally to a ligature. The wound was then closed and the rats were returned to the animal house. In the control experiments, the left sciatic nerve was laid open and the same procedure was followed, except that the nerve was left intact. Two weeks afterwards, the sciatic nerves were exposed and 1 μl of a 2% solution of a CTB-horseradish peroxidase (HRP) conjugate (Sigma) was injected into the nerves with a Hamilton microsyringe under chloral hydrate (400 mg/kg, i.p.) anaesthesia. Three days after the injection of CTB-HRP, the animals were deeply anaesthetized and perfused transcardially with an aldehyde fixative containing 1% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH = 7.4), followed by 400 ml of cold phosphate buffer containing 30% sucrose. The medulla, spinal cord segments L1–L6 and dorsal root ganglia L4–L5 were removed and stored in the sucrose buffer solution. Serial frozen sections of the dorsal root ganglia, the spinal cord and the medulla 15 or 60 μm in thickness were cut, mounted on chromalum-gelatin-coated slides and reacted for the demonstration of peroxidase activity according to Mesulam [10], using 3,3′,5,5′-tetramethylbenzidine (TMB) as chromogen. After the completion of the enzyme reaction, most slides were dried overnight and then dehydrated briefly in ethanol, cleared in xylene and mounted in Permount. Other sections were counterstained with neutral red.

Size-frequency distribution histograms of CTB-HRP-labelled neurons were generated by measuring the sizes of neurons with clear-cut nuclei in representative serial sections of dorsal root ganglion L5 of each animal by means of a light microscope equipped with a camera lucida and a digitizing tablet connected to a computerized system [6,16].

The findings on the spinal distribution of transganglionically transported CTB-HRP in the control (vehicle-treated) animals confirmed previous observations [15,20]. The injection of CTB-HRP into an intact nerve resulted in the labelling of the deeper layers of the spinal dorsal horn, but not the substantia gelatinosa (Fig. 1a). However, after the injection of the tracer into a chronically transected nerve, heavy homogeneous peroxidase staining was visualized not only in the deep dorsal horn, but also within the

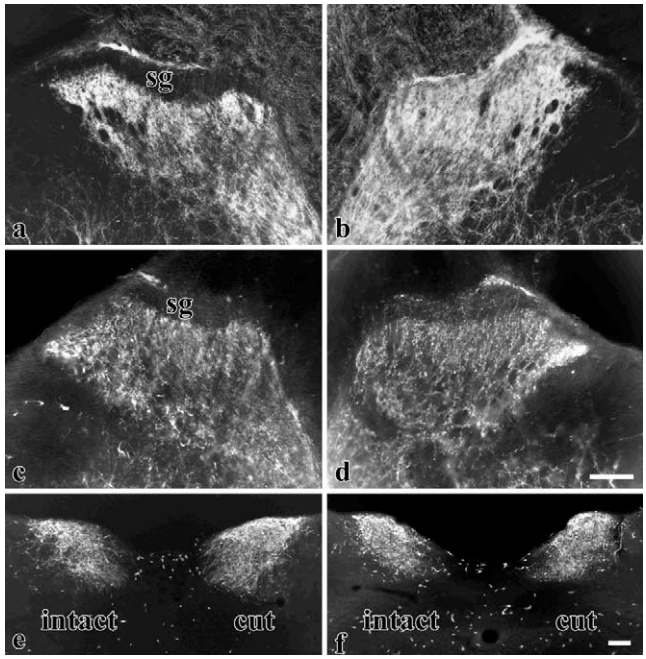


Fig. 1. Inverse microphotographs illustrating the distribution of spinal primary afferents transganglionically labelled with CTB-HRP in the spinal dorsal horn and the medulla oblongata relating to the intact (a, c) and the transected (b, d) sciatic nerves of the control (a, b, e) and the capsaicin-pretreated (c, d, f) rats. Note the lack of labelling of the substantia gelatinosa of the dorsal horn relating to the intact nerve of both the control and the capsaicin-pretreated rats. In the substantia gelatinosa and the marginal zone of the dorsal horn relating to the transected nerve, strong homogeneous labelling is seen in the control rats, whereas only faint labelling can be observed in the capsaicin-pretreated animals. sg = substantia gelatinosa. The scale bars in d and f correspond to 100 μm and apply to a–d and e–f, respectively.

substantia gelatinosa and the marginal zone (Fig. 1b). The injection of CTB-HRP into the intact sciatic nerve of the capsaicin-pretreated rats resulted solely in the labelling of the deeper layers of the dorsal horn, the substantia gelatinosa remaining free of labelling (Fig. 1c). Measurement of the maximum dorsoventral extent of the unlabelled areas of the substantia gelatinosa and the marginal zone ipsilateral to the intact sciatic nerve in 30 sections of the spinal cord of the control and the capsaicin-pretreated animals revealed no significant difference between the capsaicin-pretreated and the control rats ($n = 4$, Student's t-test). The injection of the tracer into the chronically transected nerve of the capsaicin-pretreated rats resulted in a strong labelling of the deep dorsal horn and also a faint, but distinct labelling of the substantia gelatinosa (Fig. 1d). This latter labelling was confined to a few individual nerve fibers and was much weaker than the essentially homogeneous strong labelling seen after nerve transection in the control animals (cf. Fig. 1b). In the medulla, labelling was observed in the gracile nucleus relating to both the intact and the transected nerves. In accord with previous findings [18], the intensity and extent of the labelling was increased ipsilaterally to the injured nerve (Fig. 1e). This lesion-

induced increase in labelling was also noted in the capsaicin-pretreated rats (Fig. 1f).

Light microscopy of spinal ganglion L5 and analysis of the size-frequency distribution histograms in the control rats revealed that, the CTB-HRP-labelled neurons in the ganglia relating to the intact sciatic nerve involved mostly larger ganglion cells, although a moderate proportion of small cells were also labelled (Figs. 2a and 3a). In contrast, after nerve transection, a majority of the small cells displayed peroxidase activity, indicating the presence of CTB-HRP (Figs. 2b and 3b). In agreement with earlier findings [3,4,8], neonatal treatment with capsaicin resulted in a profound reduction in the proportion of small dorsal root ganglion neurons. In the ganglia relating to the intact nerve in these rats, CTB-HRP was localized to larger cells (Figs. 2c and 3c). In the ganglia relating to the transected nerve, an increase in the proportion of labelled cells of all sizes was observed (Figs. 2d and 3d).

The present findings confirm previous reports on the transganglionic labelling of the substantia gelatinosa after the injection of CTB-HRP into injured, but not into intact peripheral nerves [1,9,12,15,16,20] and demonstrate a critical role of the capsaicin-sensitive primary sensory neurons in the mechanism of this phenomenon. Indeed, neonatal capsaicin treatment producing an irreversible loss of small sensory ganglion neurons, and consequently a selective C-fiber deafferentation of the spinal dorsal horn, prevented the lesion-induced strong homogeneous labelling of the substantia gelatinosa by CTB-HRP. Until recently, it was widely believed that the appearance of CTX-HRP labelling in the substantia gelatinosa after peripheral nerve lesions could be attributed to a sprouting response of myelinated, A-fiber afferents, which normally terminate only in the deeper layers of the dorsal horn. However, this view was recently challenged when it was shown that after nerve transection, the C-fiber primary afferents become

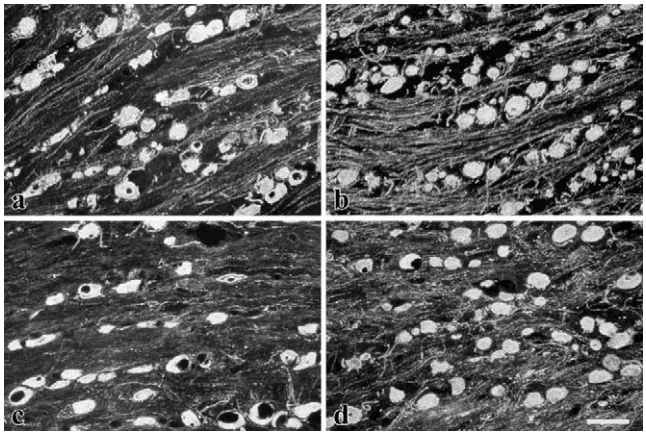


Fig. 2. Inverse microphotographs showing CTB-HRP-labelled neurons of spinal ganglion L5 relating to the intact (a, c) and the transected (b, d) sciatic nerves of the control (a, b) and the capsaicin-pretreated rats (c,d). Note the increase in number of the labelled small cells after nerve transection in the control (b), but not in the capsaicin-pretreated (d) rats. The scale bar in d corresponds to 100 μm and applies to all microphotographs.

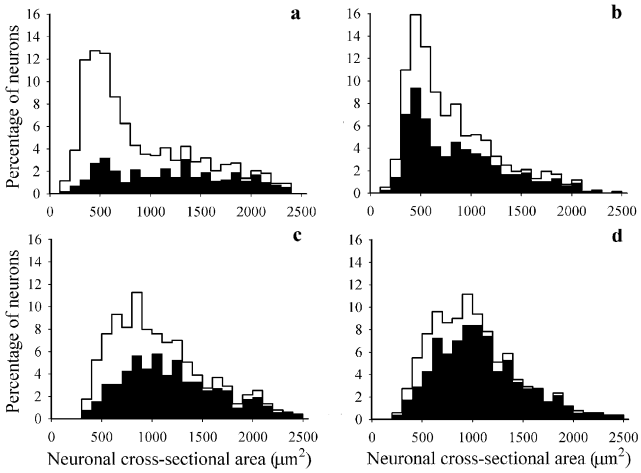


Fig. 3. Size-frequency distribution histograms of neuronal populations of dorsal root ganglion L5 relating to the intact (a, c) and the transected (b, d) sciatic nerve of the control (a, b) and the capsaicin-pretreated (c, d) rats. Clear histograms represent the total neuronal population in the ganglion, whereas overimposed filled histograms represent the CTB-HRP-labelled neurons.

capable of the uptake and transport of CTB-HRP, and that this neuroplastic change may be accounted for by a phenotypic switch of the C-fibers rather than by a sprouting response of the A-fibers [1,5,6,16–18]. The present findings lend further support to this assumption by demonstrating that, in contrast with the situation in the control animals, after the elimination of the C-fiber afferents by neonatal capsaicin treatment, the injection of CTB-HRP into the chronically transected sciatic nerve failed to produce strong homogeneous labelling of the substantia gelatinosa. These observations indicate that the presumed sprouting of the A-fibers plays little role in the lesion-induced massive transganglionic labelling of the substantia gelatinosa by CTB-HRP. The possibility that the C-fibers may be necessary for the initiation of a sprouting response in the A-fibers seems unlikely. First, electron microscopic histochemical studies furnished direct evidence of the transport of choleragenoid by unmyelinated axons in the dorsal roots relating to a transected, but not to an intact sciatic nerve [16]. Second, in capsaicin-pretreated rats, nerve transection induced an increase in the proportion of large dorsal root ganglion neurons which contained CTB-HRP reaction product, which suggests that the increased uptake and transport of CTB-HRP in the myelinated afferents is apparently independent of the C-fiber function/integrity. Finally, the increase in the intensity and extent of the choleragenoid labelling of the gracile nucleus indicates that the injury-induced increased labelling of the A-fibers occurs under conditions such that the C-fibers are missing.

The present findings raise an interesting point as concerns the demonstration of possible sprouting of the dorsal root fibers in the spinal cord. Neonatal capsaicin treatment has been shown to result not only in irreversible destruction of the C-fiber primary afferent neurons and their spinal terminations [4,8], but also in a compensatory/regenerative sprouting of the A-fiber afferents, as revealed

by both histochemical [11,14] and classical silver impregnation techniques [2]. Surprisingly, this could not be clearly observed in the present study when CTB-HRP was utilized as a neuronal tracer with the highly sensitive TMB technique for the demonstration of HRP activity; faint labelling of a few presumed sprouting A-fiber afferents was detected only after nerve transection in the capsaicin-pretreated rats. These findings indicate that the experimental conditions used in this and previous studies are unsuitable for the visualization of a true sprouting response of myelinated afferents. In the present study, the demonstration of some fiber labelling in the substantia gelatinosa of the capsaicin-pretreated animals after the injection of the tracer into the injured, but not the intact nerve may be explained by the increased uptake and transganglionic transport of CTB-HRP by injured myelinated axons, which permitted their histological visualization. The results of light microscopy and the quantitative data on the increase in the proportion of labelled large spinal ganglion cells after nerve transection support this assumption. It should be mentioned in this respect that in previous studies which showed the sprouting of myelinated afferents into the substantia gelatinosa in capsaicin-pretreated rats, HRP was either injected directly into the spinal ganglia [11] or applied to the cut central end of the dorsal root [14], presumably producing optimum labelling of the dorsal root fibers.

In conclusion, the present study has demonstrated that extensive C-fiber deafferentation of the spinal dorsal horn prevents the strong labelling of the substantia gelatinosa by CTB-HRP. These findings lend further support to the notion that peripheral nerve lesion-induced transganglionic labelling of the substantia gelatinosa by choleragenoid may be accounted for by a phenotypic switch of the C-fiber afferents, rather than by A-fiber sprouting. In addition, the results suggest that the experimental strategies for the evaluation of afferent sprouting need to be revisited and improved. Finally, the present observations may be of appreciable relevance as concerns a possible modulatory role of axonal ganglioside metabolism/content in the nociceptor function.

Acknowledgements

This work was supported in part by grants from OTKA (T-032507) and ETT (51/2000, 569/2003). The authors are grateful to Dr David Durham for linguistic revision of the manuscript and Éva Hegyeshalmi for her expert technical assistance.

References

[1] L. Bao, H.F. Wang, H.J. Cai, Y.G. Tong, S.X. Jin, Y.J. Lu, G. Grant, T. Hökfelt, X. Zhang, Peripheral axotomy induces only very limited sprouting of coarse myelinated afferents into inner lamina II of rat spinal cord, Eur. J. Neurosci. 16 (2002) 175–185.

[2] J.A. Beal, D.S. Knight, Classification of aberrant primary afferents in the substantia gelatinosa of the rat following neonatal capsaicin treatment, Neurosci. Lett. 74 (1987) 139–144.

[3] G. Jancsó, Pathobiological reactions of C-fiber primary sensory neurones to peripheral nerve injury, Exp. Physiol. 77 (1992) 405–431.

[4] G. Jancsó, E. Király, A. Jancsó-Gábor, Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones, Nature 270 (1977) 741–743.

[5] G. Jancsó, P. Sántha, Phenotypic switch of C-fiber afferents rather than A-fiber sprouting is responsible for peripheral nerve lesion-induced structural reorganisation of choleragenoid-binding primary afferents in the rat spinal dorsal horn, Neuropeptides 36 (2002) 467–468.

[6] G. Jancsó, P. Sántha, K. Gecse, Peripheral nerve lesion-induced uptake and transport of choleragenoid by capsaicin-sensitive C-fiber spinal ganglion neurons, Acta Biol. Hung. 53 (2002) 77–84.

[7] I. Kohama, K. Ishikawa, J.D. Kocsis, Synaptic reorganization in the substantia gelatinosa after peripheral nerve neuroma formation: aberrant innervation of lamina II neurons by Abeta afferents, J. Neurosci. 20 (2000) 1538–1549.

[8] S.N. Lawson, The morphological consequences of neonatal treatment with capsaicin on primary afferent neurons in adult rat, Acta Phys. Hun. 69 (1987) 315–321.

[9] H.A. Lekan, S.M. Carlton, R.E. Coggeshall, Sprouting of A beta fibers into lamina II of the rat dorsal horn in peripheral neuropathy, Neurosci. Lett. 208 (1996) 147–150.

[10] M.M. Mesulam, Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction product with superior sensitivity for visualizing neural afferents and efferents, J. Histochem. Cytochem. 26 (1978) 106–117.

[11] J.I. Nagy, S.P. Hunt, The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment, J. Comp. Neurol. 218 (1983) 145–158.

[12] S. Nakamura, R.R. Myers, Myelinated afferents sprout into lamina II of L3–5 dorsal horn following chronic constriction nerve injury in rats, Brain Res. 818 (1999) 285–290.

[13] M. Okamoto, H. Baba, P.A. Goldstein, H. Higashi, K. Shimoji, M. Yoshimura, Functional reorganization of sensory pathways in the rat spinal dorsal horn following peripheral nerve injury, J. Physiol. 532 (2001) 241–250.

[14] M. Réthelyi, M.Z. Salim, G. Jancsó, Altered distribution of dorsal root fibers in the rat following neonatal capsaicin treatment, Neuroscience 18 (1986) 749–761.

[15] B. Robertson, G. Grant, A comparison between wheat germ agglutinin-and choleragenoid-horseradish peroxidase as anterogradely transported markers in central branches of primary sensory neurones in the rat with some observations in the cat, Neuroscience 14 (1985) 895–905.

[16] P. Sántha, G. Jancsó, Transganglionic transport of choleragenoid by capsaicin-sensitive C-fiber afferents to the substantia gelatinosa of the spinal dorsal horn after peripheral nerve section, Neuroscience 116 (2003) 621–627.

[17] S.A. Shehab, R.C. Spike, A.J. Todd, Evidence against cholera toxin B subunit as a reliable tracer for sprouting of primary afferents following peripheral nerve injury, Brain. Res. 964 (2003) 218–227.

[18] Y.G. Tong, H.F. Wang, G. Ju, G. Grant, T. Hökfelt, X. Zhang, Increased uptake and transport of cholera toxin B-subunit in dorsal root ganglion neurons after peripheral axotomy: possible implications for sensory sprouting, J. Comp. Neurol. 404 (1999) 143–158.

[19] C.J. Woolf, P. Shortland, R.E. Coggeshall, Peripheral nerve injury triggers central sprouting of myelinated afferents, Nature 355 (1992) 75–78.

[20] C.J. Woolf, P. Shortland, M. Reynolds, J. Ridings, T. Doubell, R.E. Coggeshall, Reorganization of central terminals of myelinated primary afferents in the rat dorsal horn following peripheral axotomy, J. Comp. Neurol. 360 (1995) 121–134.

PAPER II

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

Neuroscience xx (2011) xxx

DISPARATE CHANGES IN THE EXPRESSION OF TRANSIENT RECEPTOR POTENTIAL VANILLOID TYPE 1 RECEPTOR mRNA AND PROTEIN IN DORSAL ROOT GANGLION NEURONS FOLLOWING LOCAL CAPSAICIN TREATMENT OF THE SCIATIC NERVE IN THE RAT

C. SZIGETI,[a,b1] P. SÁNTHA,[a1] E. KÖRTVÉLY,[b] T. NYÁRI,[c] V. J. HORVÁTH,[d] É. DEÁK,[a] M. DUX,[a] K. GULYA[b] AND G. JANCSÓ[a]*

[a]*Department of Physiology, Faculty of Medicine, University of Szeged, H-6720 Szeged, Dóm tér 10, Hungary*

[b]*Department of Cell Biology and Molecular Medicine, Faculty of Medicine and Faculty of Science and Informatics, University of Szeged, H-6720 Szeged, Somogyi u. 4, Hungary*

[c]*Department of Medical Physics and Informatics, University of Szeged, H-6720 Szeged, Korányi fasor 9, Hungary*

[d]*Second Department of Internal Medicine, University of Szeged, H-6720 Szeged, Korányi fasor 6, Hungary*

Abstract—*In situ* hybridization, quantitative reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry, and Western blot analysis were applied to study the changes in expression of the major nociceptive ion channel transient receptor potential vanilloid type 1 receptor (TRPV1) after the perineural application of capsaicin or nerve transection. In control rats, quantitative morphometric and statistical analyses of TRPV1 protein and mRNA expression in L5 dorsal root ganglion cells revealed distinct populations of small (type C) and small-to-medium (type B) neurons, which showed very high and moderate levels of TRPV1, whereas larger (type A) neurons mostly did not express this receptor. After either transection or capsaicin treatment of the sciatic nerve, immunohistochemistry and Western blotting demonstrated a massive (up to 80%) decrease in the proportion of TRPV1-immunoreactive neurons and TRPV1 protein at all postoperative survival times. *In situ* hybridization indicated marked decreases (up to 85%) in the proportion of neurons that expressed TRPV1 mRNA after sciatic nerve transection. In contrast, although perineural treatment with capsaicin resulted in similar substantial decreases in the proportions of type B and C neurons of the L5 dorsal root ganglia 3 days postoperatively, a clear-cut tendency to recovery was observed thereafter. Hence, the proportions of both type B and C neurons expressing TRPV1 mRNA reached up to 70% of the control levels at 30 days postoperatively. In accord with these findings, quantitative RT-PCR revealed a marked and significant recovery in TRPV1 mRNA after perineural capsaicin but not after nerve transection. These observations suggest the involvement of distinct cellular mechanisms in the regulation of the TRPV1 mRNA expression of damaged neurons, specifically triggered by the nature of the injury. The present findings imply that the antinociceptive and anti-inflammatory effects of perineurally applied capsaicin involve distinct changes in neuronal TRPV1 mRNA expression and long-lasting alterations in (post)translational regulation.

Key words: capsaicin, perineural, TRPV1 mRNA, pain, nerve transection, *in situ* hybridization.

Chemosensitive primary sensory neurons, which are sensitive to capsaicin (Jancsó, 1968; Jancsó et al., 1977) and express the transient receptor potential vanilloid type 1 receptor (TRPV1) (Caterina et al., 1997; Caterina and Julius, 2001), play a fundamental role in pain mechanisms. By virtue of their dual functional character, these particular nociceptive neurons comprise a unique population of primary afferent neurons, which transmit impulses generated by noxious stimuli and release neuropeptides from their peripheral and central terminals in response to stimulation (Maggi and Meli, 1988; Holzer, 1991; Jancsó, 2009). The chemosensitive primary afferent neurons, which are selectively sensitive to the stimulatory and neurotoxic effects of capsaicin, account for around 50% of the dorsal root ganglion (DRG) cells and 95% of the unmyelinated dorsal root fibers in the rat (Jancsó et al., 1977, 2011; Nagy and Hunt, 1983). Previous studies have demonstrated that selective elimination of these nociceptive afferents either from the whole animal or from selected regions of the body by the systemic (neonatal) or localized (perineural) administration of capsaicin and related vanilloids leads to profound antinociceptive and anti-inflammatory effects (Jancsó et al., 1977, 1980; Fitzgerald and Woolf, 1982; Gamse et al., 1982). The perineural application of vanilloid compounds that results in highly selective regional thermal and chemical analgesia has attracted much interest because of the promising therapeutic relevance of this intervention. Local application of capsaicin or resiniferatoxin has been shown to induce long-lasting increases in the thresholds of nociceptive responses elicited by chemical irritants and intense heat stimuli (Jancsó et al., 1980; Gamse et al., 1982; Chung et al., 1985; Kissin et al., 2002). Local treatment with capsaicin or resiniferatoxin also reduces inflammatory

[1] These authors contributed equally to the article.

*Corresponding author. Tel: +36-62-545099; fax: +36-62-545842.
E-mail address: jancso@phys.szote.u-szeged.hu or gaborjancso@yahoo.co.uk (G. Jancsó).

Abbreviations: B2-MG, beta-2-microglobulin; CSA, cross-sectional area; DRG, dorsal root ganglion; GV, gray value; NGF, nerve growth factor; PBS, phosphate buffered saline; ROC, receiver operating characteristic; ROD, relative optical density; RT-PCR, reverse transcription polymerase chain reaction; TBS, Tris-buffered saline; TRPV1, transient receptor potential vanilloid type 1 receptor.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.10.058

thermal and mechanical hyperalgesia and ischemic reactive hyperemia (Kissin et al., 2002; Domoki et al., 2003; Pospisilova and Palecek, 2006; Holzer, 2008; Jancsó et al., 2008; Oszlács et al., 2009) and arthritis (Donaldson et al., 1995). Antidromic vasodilatation and neurogenic inflammation, the cardinal local vascular responses of chemosensitive afferent endings brought about through stimulation with chemical irritants or antidromic stimulation of sensory nerves, are completely abolished by such treatment (Jancsó and Király, 1980; Oszlács et al., 2009). Although the antinociceptive and anti-inflammatory effects of locally applied vanilloid compounds have been repeatedly demonstrated, the mechanisms of these unique antinociceptive/analgesic effects are still unclear. Electrophysiological studies have revealed a selective and long-lasting reduction of impulse conduction in unmyelinated, but not in myelinated sensory axons (Jancsó and Such, 1983; Baranowski et al., 1986; Pini et al., 1990), associated with a reduction of polymodal nociceptor units in rat (Welk et al., 1983; Pini et al., 1990), guinea pig, and rabbit (Baranowski et al., 1986) peripheral nerves. Similar findings have been reported in monkeys following the treatment of peripheral nerves with capsaicin (Chung et al., 1993). Morphological investigations have disclosed a substantial, but partial reduction in the number of unmyelinated sensory (Baranowski et al., 1986; Jancsó and Lawson, 1990), but not autonomic (Jancsó et al., 1987) axons in capsaicin-treated peripheral nerves and in skin areas innervated by a capsaicin-treated peripheral nerve (Jancsó et al., 1980; Dux et al., 1999). Recent findings indicated that the application of resiniferatoxin to peripheral nerves induced lasting analgesia without noticeable fine structural alterations in the rat (Kissin et al., 2002, 2007). Histochemical and immunohistochemical studies have revealed the marked depletion of sensory neuropeptides from the spinal ganglia and the dorsal horn of the spinal cord relating to the peripheral nerve treated with a vanilloid agent (Gamse et al., 1982; Jancsó and Lawson, 1988; Oszlács et al., 2009). However, changes in the expression of the TRPV1, a molecular integrator of nociception (Winter et al., 1988; Caterina et al., 1997; Tominaga et al., 1998), which confers capsaicin (vanilloid) sensitivity on chemosensitive primary afferent neurons (Winter et al., 1988; Caterina et al., 1997; Michael and Priestley, 1999) have not been investigated so far after perineural treatment with vanilloid compounds. The present experiments were therefore initiated in an attempt to make use of *in situ* hybridization, quantitative reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry, and Western blot analysis to reveal possible changes in the expression of the TRPV1 following perineural capsaicin treatment and, for comparison, peripheral nerve transection.

EXPERIMENTAL PROCEDURES

Adult male Wistar rats weighing 240–260 g at the start of the experiments were used in this study. The animal house was maintained under a 12-h light/dark cycle. All experimental procedures were approved by the Ethical Committee for Animal Care of the University of Szeged and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

Perineural capsaicin treatment

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p., Reanal, Budapest, Hungary). The sciatic nerves were exposed high in the thigh on both sides, and small pieces of gelfoam moistened with 0.1 ml of a 1% solution of capsaicin (Fluka, Buchs, Switzerland) or the same volume of the vehicle (6% ethanol, 8% Tween 80 in saline) were wrapped around the right and left nerves, respectively. After 20 min, the gelfoam pieces were removed, the wounds were closed, and the rats were returned to the animal house. After 3, 14, or 30 days, the animals were again anesthetized and sacrificed for immunohistochemical and *in situ* hybridization analyses.

Peripheral nerve transection

The rats were anesthetized with chloral hydrate (400 mg/kg, i.p., Reanal, Budapest, Hungary). The right sciatic nerve was exposed high in the thigh and transected distal to a ligature. Sham-operated animals served as controls. After 3, 14, or 30 days, the animals were again anesthetized and sacrificed for immunohistochemical and *in situ* hybridization analyses.

In situ hybridization

The synthesis of the cRNA probe and *in situ* hybridization were carried out as described by Maniatis et al. (1982), with slight modifications. To generate TRPV1 mRNA-specific probes, total mRNA was isolated from rat trigeminal ganglia and was reverse transcribed by using the universal dT17-adapter primer (5'-GACTCGAGTCGAGTCGACATCGATTTTTTTTTTTTTTTT-3', M-MuLV reverse transcriptase; Fermentas, Vilnius, Lithuania) according to the manufacturer's recommendations. This cDNA template was used to perform RT-PCR with the following primer combination: forward 5'-AACCATGGAACAACGGGCTAGC-3'; reverse 5'-AACTCGAGTTAGAACAGAGCTGACA-3'. The amplified 255 bp length product was cloned into pcDNA3 vector (Invitrogen, Carlsbad, CA, USA). The identity of the amplified product was confirmed by DNA sequencing and Northern blotting. After linearization of the vectors, sense and antisense digoxigenin-11-UTP-labeled cRNA probes were transcribed with T7 or SP6 polymerases, using a DIG RNA labeling kit (Boehringer Mannheim, Mannheim, Germany) according to the manufacturer's protocol.

For *in situ* hybridization, DRGs were quickly removed, embedded in Cryomatrix embedding material (Shandon Scientific, Pittsburgh, PA, USA), and frozen immediately at −70 °C. Serial frozen sections of DRGs (15 μm in thickness) were cut on a cryostat and thaw-mounted onto 3-aminopropyltriethoxysilane-coated glass slides. Sections were air-dried and stored at −20 °C until further processing. The specimens were fixed for 5 min in 2× sodium chloride–sodium citrate (SSC) buffer (0.3 M NaCl and 0.03 M Na-citrate, pH 7.0) containing 4% formaldehyde, washed twice in 2× SSC buffer for 2 min, permeabilized with 0.1% Triton X100, washed again as before, and then rinsed in 0.1 M triethanolamine containing 0.25% acetic anhydride at room temperature for 5 min. Hybridization was performed in 50 μl hybridization solution (50% formamide, 5× sodium chloride–sodium phosphate–EDTA buffer, 1× Denhardt's reagent, 10% dextran sulfate, 50 mM dithiothreitol, 100 μg/ml salmon sperm DNA, and 100 μg/ml yeast tRNA containing 200 nmol/ml labeled probe) under parafilm cover slips in a humidified chamber at 56 °C for 20 h. The sections were extensively rinsed in 2× SSC buffer supplemented with 50% formamide at 50 °C for 15 min, treated with RNase A at 37 °C for 30 min, and washed again in 2× SSC–50% formamide solution at 50 °C. To block nonspecific antibody binding, sections were incubated with

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

buffer 1 (100 mM Tris–HCl and 150 mM NaCl, pH 7.5) containing 5% normal goat serum for 1 h at room temperature, followed by incubation with alkaline phosphatase-conjugated anti-digoxigenin antibody (1:2500, Boehringer Mannheim GmbH, Mannheim, Germany) in buffer 1 at 4 °C overnight. Sections were washed in buffer 1 for 3×5 min, rinsed in buffer 2 (100 mM Tris–HCl, 100 mM NaCl, and 50 mM $MgCl_2$, pH 9.5) for 10 min, and developed in buffer 2 containing 340 μg/ml nitro blue tetrazolium and 180 μg/ml 5-bromo-4-chloro-3-indolyl phosphate for 12 h in a dark chamber. The reaction was terminated by rinsing the slides in a buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0) for 10 min. The sections were covered with glycerol.

Quantitative RT-PCR measurements

To measure changes in the total TRPV1 mRNA expression in DRGs affected by the transection or capsaicin treatment of the sciatic nerve, quantitative RT-PCR was used. Rats were terminally anesthetized 3, 14, and 30 days after surgery, and the L5 DRGs were excised and transferred into 1 ml ice-cold Trizol reagent (Invitrogen, Carlsbad, CA, USA). Total mRNA was isolated by Trizol solution according to the protocol of the manufacturer. The extracted total mRNA was reverse transcribed by using BioRad iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA). Specific primers were designed to amplify TRPV1 and beta-2-microglobulin (B2-MG, reference gene) by using the Primer-Blast open source software (http://www.ncbi.nlm.nih-.gov/tools/primer-blast). The sequences of the primers were as follows: B2-MG (NM_012512; reference gene): 5′-TCTCCG-GTGGATGGCGAGAGT-3′ (reverse); 5′-GCTCGCTCGGT-GACCGTGATC-3′ (forward); TRPV1 (NM_031982.1): 5′-TGTCTTCCGGGCAACGTCCA-3′ (reverse); 5′-AAGCGCCT-GACTGACAGCGA-3′ (forward). Primers were synthesized by Integrated DNA Technologies (Leuven, Belgium). These primers produced distinct PCR amplification products with length of 129 bp for TRPV1 and 106 bp for B2-MG, as confirmed by gel-electrophoresis. Quantitative RT-PCR was performed in triplicates utilizing SYBR Green technique (iQ SYBR Green Supermix, Bio-Rad, Hercules, CA, USA) and BioRad MyiQ5 Real Time Detection System running the following amplification protocol: 10 min on 95 °C (hot start) followed by 40 amplification cycles (denaturation: 10 s on 95 °C, annealing: 30 s on 56 °C; elongation and detection: 20 s on 72 °C). At the end of the amplification, melt-curve analysis was also applied to exclude nonspecific fluorescent signals. Relative quantities of target (TRPV1) mRNAs as compared with the housekeeping reference gene B2-MG were calculated by using the Pfaffl-method (Pfaffl, 2001).

TRPV1 immunohistochemistry

The animals were deeply anesthetized and perfused transcardially with an aldehyde fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The L5 DRG was removed and postfixed in the same fixative for 2 h and then placed into a phosphate-buffered 30% sucrose solution. Representative serial sections of L5 DRGs 15 μm in thickness were cut on a cryostat and mounted on gelatin-coated glass slides. Sections were rinsed twice in phosphate-buffered saline (PBS) and incubated overnight with the primary antibody (1:1000; rabbit anti-TRPV1 IgG, ACC030, Alomone Labs, Jerusalem, Israel) with 0.3% Triton X100 added. After rinsing in PBS, the sections were incubated for 2 h with the secondary antibody (1:500 biotin-conjugated donkey anti-rabbit IgG, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) diluted in PBS containing 0.3% Triton X100. To visualize the biotin-conjugated antibody, the sections were rinsed and treated with the Vectastain ABC Elite staining kit (Vector laboratories, Burlingame, CA, USA) according to the instructions of the manufacturer. The sections were dehydrated and covered with DPX mounting medium (Fluka, Buchs, Switzerland).

Semiquantitative densitometry

The sections cut from the DRGs and processed for visualization of the TRPV1 mRNA by *in situ* hybridization or the TRPV1 protein by immunohistochemistry were examined under bright-field illumination with a DMLB microscope (Leica, Wetzlar, Germany) equipped with a Nikon Coolpix (Nikon, Tokyo, Japan) digital camera. Under identical conditions, microphotographs were taken of DRGs relating to control sciatic nerves and sciatic nerves transected or treated perineurally with capsaicin following a systemic random sampling method. The optical density of DRG neurons with clear-cut nuclei was measured by means of the NIH Scion Image analysis program. In sections processed for the demonstration of TRPV1 mRNA, many neurons exhibited granular staining of different intensities in the perikaryon. In contrast, in labeled neurons the TRPV1 immunoreactivity displayed diffuse staining throughout the cell bodies and sometimes in their axons. Gray values (GVs) between 0 and 255 were assigned to each neuron with a clearly visible nucleus, and their cross-sectional areas (CSAs) were measured. Relative optical densities (RODs) were determined according to the equation $ROD = \log_{10}(255/(255 - GV))$. The CSA and ROD for each cell were determined and plotted as distribution histograms or scatter plots.

Classification of DRG neurons

The DRG neurons were classified into different subpopulations by using a statistical approach. Pilot experiments suggested the existence of three distinct neuronal subpopulations in the control DRGs, with different levels of mRNA signal and TRPV1 immunostaining. Discriminant analysis was performed to define the ROD classification effect among the different subpopulations of DRG neurons. To determine the threshold values of ROD for the separation of the neuronal subpopulations, the receiver operating characteristic (ROC) method was applied pairwise (Armitage, 2001; Armitage and Colton, 2005).

Western blot analysis

L5 DRGs were removed from rats 3, 14, and 30 days after perineural capsaicin treatment or transection of the sciatic nerves and were homogenized immediately in ice-cold radio immuno precipitation assay (RIPA) buffer containing 50 mM Tris (pH 8), 150 mM sodium chloride, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 2 μg/ml leupeptin (Sigma), and 1 μg/ml pepstatin (Sigma-Aldrich, St. Louis, MO, USA). The homogenates were centrifuged at 15000 g for 10 min. The pellet was discarded, and protein concentrations from the supernatant were determined according to the method of Lowry et al. (1951). Protein samples (60 μg/well) were separated through a 12% SDS-polyacrylamide gel and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and blocked for 12 h in 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20. The membranes were incubated for 2 h with rabbit anti-TRPV1 (1:500, Chemicon, Temecula, CA, USA) and mouse anti-β-actin primary antibody (1:20000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) in 1% nonfat dry milk in 0.1% TBS–Tween 20. After three washes in 0.1% TBS–Tween 20, the membranes were incubated for 1 h with the appropriate peroxidase-conjugated secondary antibodies (1:2000, Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK), and washed five times as before. The enhanced chemiluminescence method (ECL Plus Western blotting detection reagent; Amersham Biosciences, Little Chalfont, UK) was used to reveal immunoreactive bands according to the manufacturer's protocol. The films were scanned at 600×600 dpi resolution, and the densitometric quantification was performed by the ImageJ public domain image processing and analysis software (NIH, Bethesda, MD, USA). After subtracting background, TRPV1 band

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

densities were normalized to β-actin. The ratio of the TRPV1 to β-actin band density was used to calculate the changes in TRPV1 expression. Results of three independent experiments are shown as means±SD.

Statistics

The experimental data are shown as means±SD. Statistical analyses were performed with ANOVA and Holm-Sidak, Brown-Forsythe, or Bonferroni correction methods for *post hoc* comparisons by using SPSS (v.18, Statistical Software package, IBM Corporation, NY, USA). Differences between groups were considered statistically significant if $P<0.05$.

RESULTS

Localization of TRPV1 mRNA and protein in the L5 DRG of the rat

In the control DRGs, three types of neurons could be distinguished with different levels of TRPV1 mRNA expression and TRPV1-immunostaining. Small- to medium-sized neurons displayed intense and moderate expression levels, whereas particularly the larger neurons were mostly devoid of TRPV1 mRNA and protein (Fig. 1A–D). The optimal cut-off point for the TRPV1 mRNA ROD to distinguish between group C and the remaining population was 0.40, which provided a specificity of 96% and a sensitivity of 90%. Similarly, a cut-off value of 0.24 provided the optimal differentiation between groups A and B (Fig. 1E, G). Type C and B neurons were characterized by their small (CSA range: 0–400 μm^2) and medium sizes (range: 410–900 μm^2), and high (0.41–1) and moderate (0.25–0.40) RODs, respectively. The population of type A neurons was composed of cells of various sizes with low RODs (0–0.24), which hardly exceeded the background ROD. The type C and B neurons were regarded as expressing high and moderate levels of TRPV1 mRNA, whereas type A cells were classified as TRPV1-negative neurons. The *in situ* hybridization experiments revealed that around half of the DRG cells expressed TRPV1 mRNA in control ganglia. The type C cells accounted for around 19% and the type B cells approximately 29% of the total neuronal population. About half (51%) of the cells in the DRGs were clearly negative for TRPV1 mRNA. Although the majority of the TRPV1 mRNA-negative neurons were large, some small neurons also exhibited low RODs.

Statistical analysis of the TRPV1-immunopositive neurons revealed three subpopulations of DRG neurons with respect to their TRPV1 protein content (Fig. 1F, H): the type C and B neurons were mainly small to medium-sized, with strong or moderate staining intensity, respectively, whereas the TRPV1-negative neurons were mostly large.

Effects of perineural capsaicin treatment or transection of the sciatic nerve on the expression of the TRPV1 in the L5 DRG of the rat

In the rat, the sensory fibers of the sciatic nerve originate from the fourth, the fifth, and (to a much lower extent) the sixth lumbar DRGs (Green, 1968). Up to 85% of the neurons in the fifth lumbar DRG project their axons into the sciatic nerve (Yip et al., 1984; Aldskogius et al., 1988). In the present study, therefore, the fifth lumbar DRG was chosen to study possible changes in the expression of the TRPV1 following two types of nerve injury: nerve transection, a physical injury resulting in neurotmesis, damage to all types of axons of the sciatic nerve (Seddon, 1943), and perineural treatment with capsaicin, which produces a selective chemodenervation of C-fiber afferents, but leaves the continuity of the nerve intact.

Perineural treatment with capsaicin resulted in a rapid decrease in the expression of TRPV1 mRNA in the neurons of the fifth lumbar DRG, with reductions by about 50% and 75% in type B and C cells 3 days after the treatment. However, this initial decrease in TRPV1 expression was followed by a distinct recovery and the proportion of TRPV1 mRNA-expressing neurons gradually increased up to 70% of the control levels toward the end of the study (Table 1). The experiments using quantitative RT-PCR confirmed these findings by showing an early and marked reduction in TRPV1 mRNA expression already 3 days after perineural capsaicin treatment. However, at later survival times quantitative RT-PCR measurements revealed a clear-cut tendency to recovery toward control expression levels resulting in a marked and statistically significant increase in TRPV1 mRNA at 30 days (Fig. 2A). Study of the localization of the TRPV1 protein by means of immunohistochemistry revealed that the proportion of TRPV1-positive ganglion cells had decreased markedly (to about 30% of the control level) 3 days after perineural capsaicin treatment, and it remained at that low level throughout the entire period of the study (Table 1). The reduction in the proportion of type C cells was especially pronounced, by about 85%. The analysis of the experimental data clearly showed the time-dependent and cell type-specific changes in the expression of TRPV1 mRNA and protein, respectively (Figs. 3 and 4). Western blot analysis of the TRPV1 protein supported the immunohistochemical findings. The TRPV1 protein was markedly and significantly reduced at all time points after perineural treatment with capsaicin (Fig. 2B, C).

Similarly to perineural treatment with capsaicin, peripheral nerve transection resulted in rapid and marked reductions in both TRPV1 mRNA expression and TRPV1 protein in the type B and C cells of the related fifth lumbar DRG 3 days after surgery. However, in contrast with capsaicin treatment, the TRPV1 mRNA expression did not recover, but remained at a low level for the entire remainder of the study period. In accord with this, the proportion of TRPV1-immunoreactive neurons dropped to about 30% of the control level and then remained low throughout the study. Again, the decreases in TRPV1 mRNA expression and TRPV1 protein (by about 80%) were especially marked in the type C cells (Table 1, Fig. 5). In accordance with the results obtained with *in situ* hybridization, quantitative RT-PCR measurements revealed marked and significant reductions in the TRPV1 mRNA expression 3 and 14 days after nerve transection. TRPV1 mRNA expression showed some increase after 30 days, but that did not reach significance (Fig. 2A).

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

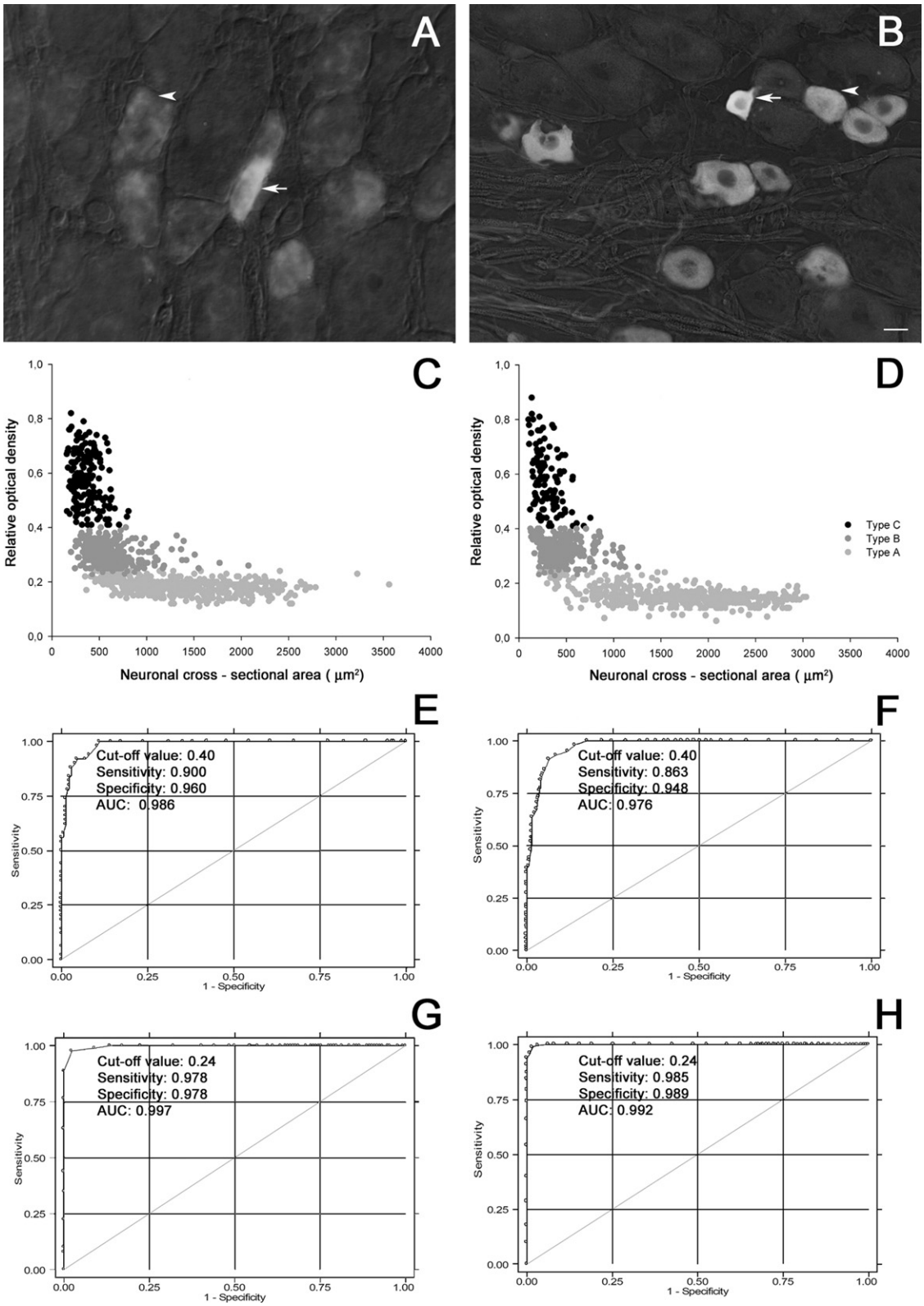


Fig. 1. (A, B) In control ganglia, *in situ* hybridization (A) and immunohistochemistry (B) revealed small- to medium-sized neurons with intense (arrow) and moderate (arrowhead) levels of TRPV1 mRNA and protein, respectively. Larger neurons were usually devoid of both TRPV1 mRNA and protein. Inverse microphotographs; scale bar indicates 25 μm. (C, D) Scatter plots of DRG cells, showing the cell sizes and the three separate populations of neurons with intense, moderate, and very low RODs. (E, G) ROC analysis of TRPV1 mRNA RODs revealed the cut-off values for the separation of type C and B (E) and type B and A (G) neurons, respectively, and disclosed the high sensitivity and specificity of the analysis involving the use of ROD. (F, H) ROC analysis of the RODs of TRPV1-immunopositive neurons revealed the cut-off values for the separation of type C and B (F) and type B and A (H) neurons, respectively, and disclosed the high sensitivity and specificity of the analysis using ROD.

Table 1. Percentage distribution of TRPV1-expressing (type B, C) and TRPV1-negative (type A) L5 DRG neurons 3, 14, and 30 d after perineural capsaicin treatment and nerve transection

Neuron type	TRPV1 mRNA expression				TRPV1 immunohistochemistry			
	Control	3 d	14 d	30 d	Control	3 d	14 d	30 d
Perineural capsaicin								
C	19±1.28	5±0.74*	9±0.62*#	12±1.4*#	17±1.73	2±0.56*	4±0.30*	3±0.43*
B	29±2.33	15±1.03*	15±0.91*	20±1.2*#	36±1.00	9±2.23*	11±0.72*	12±0.82*
A	51±3.13	79±1.76*	75±1.51*	69±4.7*#	46±1.00	89±2.06*	84±0.45*	84±0.44*
Nerve transection								
C	18±1.37	3±0.36*	2±0.03*	2±0.20*	15±1.73	2±0.60*	5±1.03*	4±0.26*
B	28±3.10	15±1.03*	16±1.72*	16±1.46*	37±4.70	16±1.90*	16±1.20*	18±0 65*
A	53±1.85	82±1.33*	82±1.69*	81±1.26*	50±0.60	80±2.35*	78±0.80*	77±1 00*

Data are expressed as means±SD.

* Significantly different from the control, $P<0.05$.

Significantly different from the 3 d value, $P<0.05$.

DISCUSSION

Chemosensitive primary sensory neurons which express the TRPV1 play a fundamental role in the transmission of nociceptive impulses (Jancsó et al., 1977; Caterina et al., 1997; Julius and Basbaum, 2001). The level of expression of the TRPV1 is an important determinant of the nociceptor function. Increases in TRPV1 mRNA expression and in peripherally directed axonal transport of TRPV1 protein have been demonstrated to be associated with neuropathic pain states and inflammation (Tohda et al., 2001). Conversely, knockdown of the TRPV1 gene prevents the development of inflammatory hyperalgesia in the rat (Caterina et al., 2000; Davis et al., 2000; Kasama et al., 2007). Hence, TRPV1 antagonism or procedures, which inhibit the activation of the receptor may produce significant antinociception. Indeed, the local application of capsaicin and some other vanilloids directly onto peripheral nerve trunks has been shown to provide long-lasting and selective chemical and thermal analgesia, confined to the region innervated by the affected nerve (Jancsó et al., 1980, 2008, 2011; Gamse et al., 1982; Fitzgerald and Woolf, 1982; Kissin et al., 2002; Knotkova et al., 2008). Despite numerous investigations that have made use of perineural capsaicin treatment (Gamse et al., 1982; Gibson et al., 1982; Chung et al., 1985; Jancsó and Lawson, 1987, 1990; Jancsó et al., 1987; Pini et al., 1990; Jancsó and Ambrus, 1994; Kissin et al., 2002), the mechanism of analgesia induced by perineural capsaicin remained unclear.

In the present study, the cell size and the ROD of the mRNA signal and the immunostaining were measured, and a statistical approach was applied to classify subpopulations of DRG neurons which express the TRPV1. In agreement with the findings of a previous radioactive *in situ* hybridization study (Michael and Priestley, 1999), the present findings revealed two subpopulations of small- and medium-sized neurons that exhibited moderate and high intensities of TRPV1 mRNA expression and TRPV1 immunoreactivity. The two populations of DRG neurons that expressed TRPV1 mRNA or TRPV1 protein could be clearly distinguished through a statistical approach involving ROC analysis based on two characteristic traits of TRPV1-positive neurons: the cell size and the ROD of the mRNA signal or the immunostaining for TRPV1. The quantitative data demonstrated that a distinct subpopulation of small DRG neurons displayed a significantly higher TRPV1 mRNA expression than did a larger population of small- and medium-sized TRPV1-expressing neurons, which accounted for around 19% and 30% of the total neuronal population, respectively, in the L5 DRGs of the rat.

The main finding of the present study is the demonstration of disparate changes in the expression of TRPV1 mRNA and protein in DRG neurons after selective chemical denervation by perineural capsaicin treatment. Further, the findings also indicate differences in the regulation of TRPV1 expression following selective chemical and physical injuries inflicted upon primary sensory neurons.

In accord with previous reports, peripheral nerve transection resulted in a substantial reduction in the proportion of TRPV1 mRNA-expressing neurons, which was already evident 3 days after surgery and persisted for at least 4 weeks in the L5 DRGs. This was closely paralleled by a significant and persistent decrease in the proportions of TRPV1-immunoreactive neurons in the L5 DRGs. These findings corroborate and extend previous reports of parallel reductions in TRPV1 mRNA expression and protein level in axotomized DRG neurons (Michael and Priestley, 1999). The present study further supported these observations by measurements of TRPV1 mRNA and protein using quantitative RT-PCR and Western blotting, respectively. The results indicated marked, significant, and permanent reductions in TRPV1 protein confirming the immunohistochemical analysis. TRPV1 mRNA expression was markedly reduced 3 and 14 days after nerve transection, but it showed a moderate increase after 30 days, which did not reach significance.

In sharp contrast, following perineural treatment with capsaicin, neurons in the L5 DRG exhibited distinct changes in TRPV1 mRNA and protein expression and TRPV1 immunostaining. Although the expression of TRPV1 mRNA in type C neurons was markedly decreased 3 days after the treatment, there was a clear-cut tendency toward recovery after 2 weeks, and a statistically significant recovery to about 60%

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

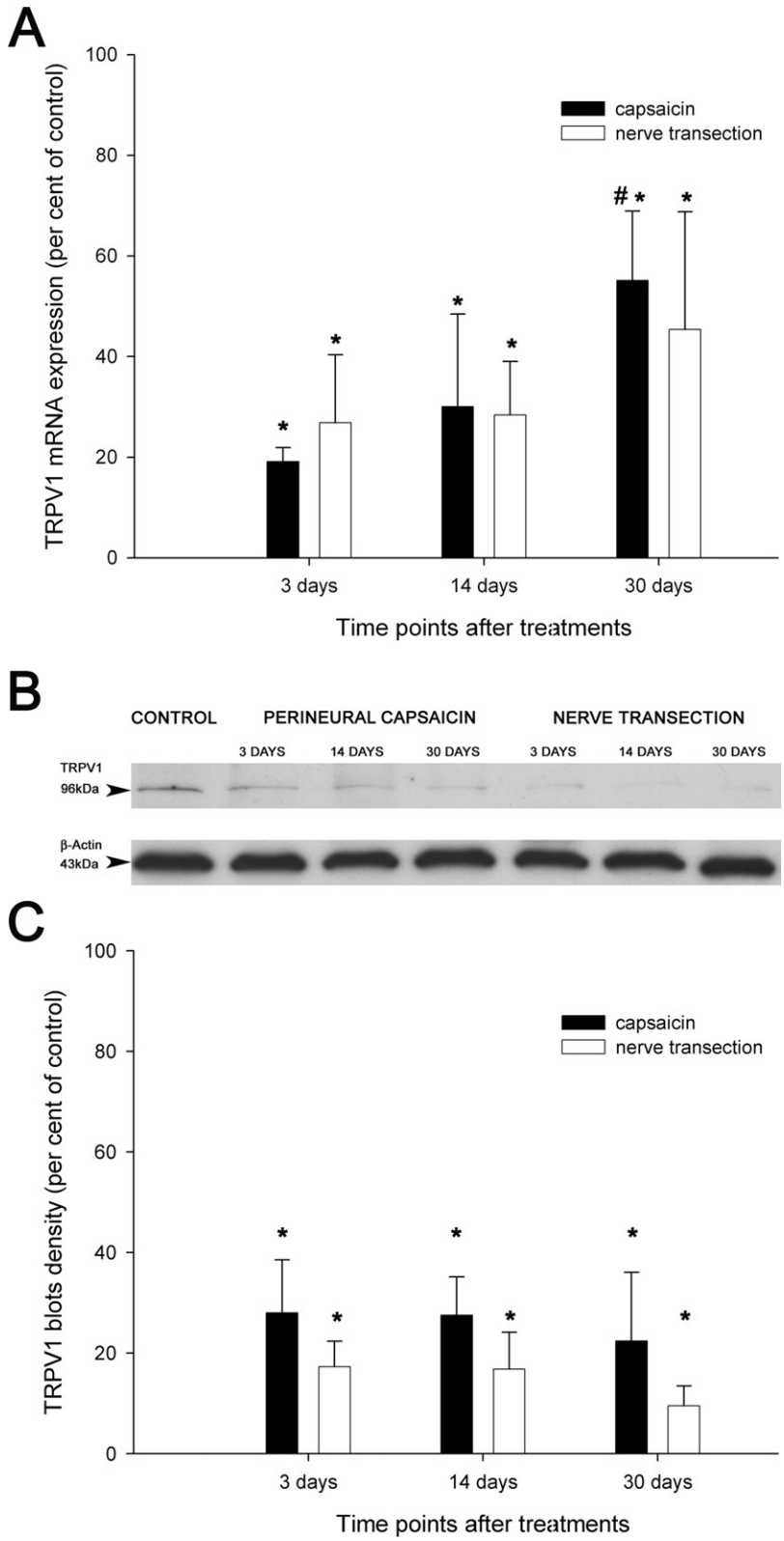


Fig. 2. Quantitative RT-PCR and Western blot analyses of the TRPV1 mRNA and protein expression. (A) Results of three to six independent experiments demonstrate the time course of changes in TRPV1 mRNA expression measured with quantitative RT-PCR in L5 DRGs following perineural capsaicin treatment and transection of the sciatic nerve. Note the marked time-dependent increase in TRPV1 mRNA expression following perineural capsaicin treatment. (B) Representative immunoblots of TRPV1 and β-actin proteins in L5 DRGs 3, 14, and 30 d after perineural capsaicin treatment and transection of sciatic nerve. (C) Results of three independent experiments demonstrate the time course of changes in TRPV1 protein. Note the marked decreases in the TRPV1 protein at all time points after perineural capsaicin treatment and nerve transection. * Significantly different from the control, $P<0.05$. # Significantly different from the 3-day value, $P<0.05$.

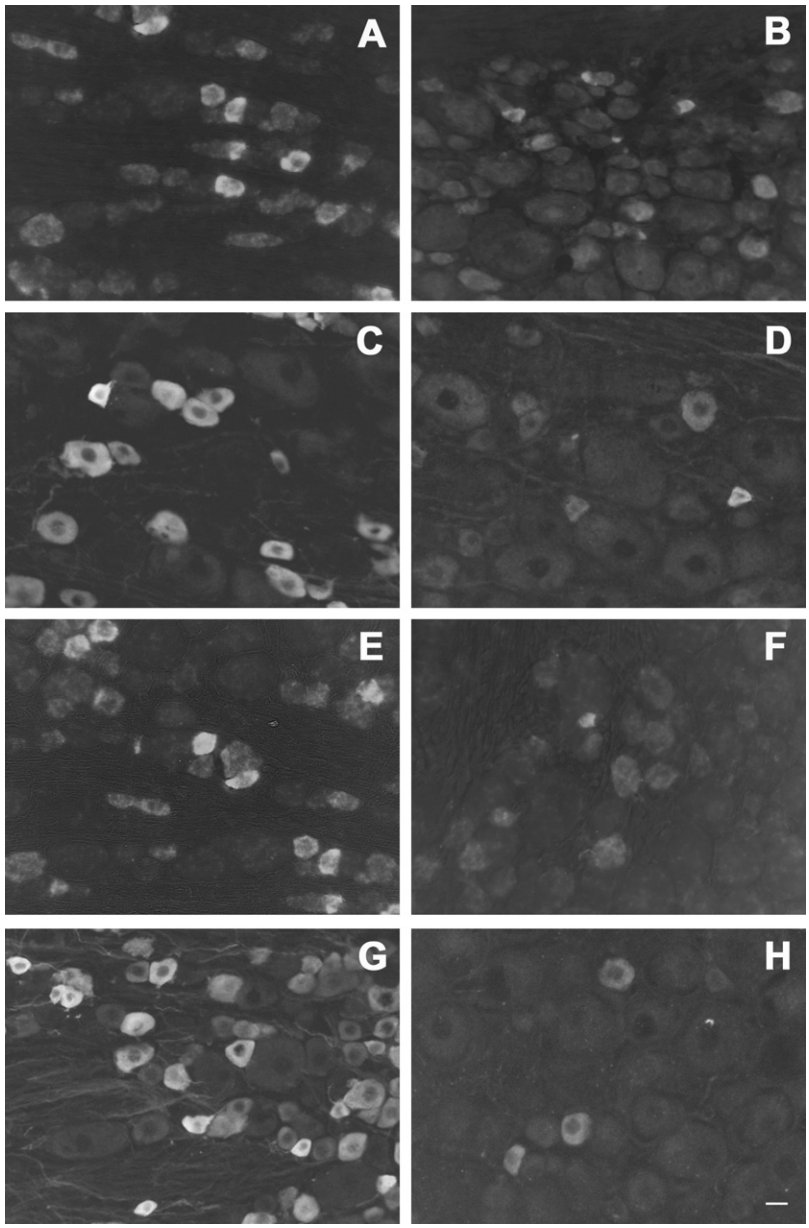


Fig. 3. Representative inverse microphotographs of the L5 DRGs, illustrating the effects of perineural capsaicin treatment (A–D) or sciatic nerve transection (E–H) on the TRPV1 mRNA expression (A, B and E, F) or TRPV1 immunoreactivity (C, D and G, H). Microphotographs illustrate the corresponding control (A, C, E, G) DRGs and DRGs ipsilateral to the sciatic nerve treated with capsaicin (B, D) or peripheral nerve transection (F, H) after 14 d. Scale bar indicates 25 μm and applies to all microphotographs.

of the control value was evident after a survival period of 4 weeks. In type B neurons, the TRPV1 mRNA expression already displayed a significant reduction by 3 days, with a significant recovery at the end of the study period. The measurements of total TRPV1 mRNA with quantitative RT-PCR in DRGs relating to the capsaicin-treated sciatic nerve confirmed these findings. An early profound decrease in TRPV1 mRNA expression was followed by a clear-cut tendency to recovery resulting in a significant increase in TRPV1 mRNA expression to about 60% of the control at the end of the study. Interestingly, however, when the TRPV1 immunoreactivity was investigated, a tendency to recovery was not observed. The proportions of TRPV1-immunoreactive type C and type B DRG neurons decreased to about 12% and 25% of the total control neuronal population after 3 days and remained at these low levels even after a survival period of 4 weeks. It should be noted that these changes in the proportions of affected TRPV1 mRNA-expressing and TRPV1-immunoreactive neurons should be considered in light of the fact that about 20% of the neurons in the L5 DRGs are not affected by the lesions for their axons run in nerves other than the sciatic nerve (Yip et al., 1984; Aldskogius et al., 1988). These immunohistochemical findings were strongly supported by measurements of the

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

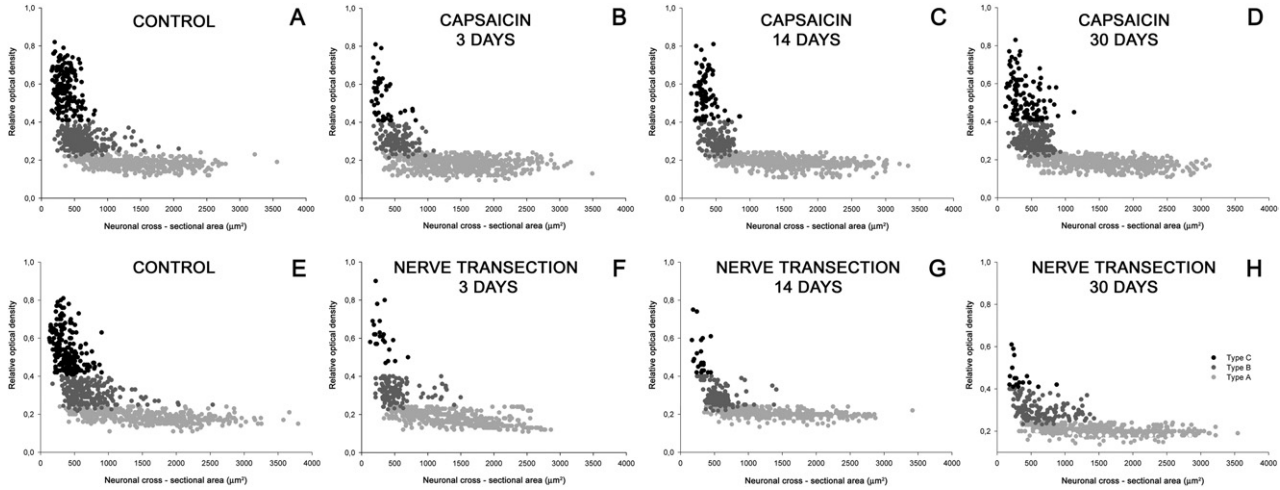


Fig. 4. Scatter plots showing the time course of changes in the populations of TRPV1 mRNA-expressing L5 DRG neurons following perineural capsaicin treatment (A–D) and transection of the ipsilateral sciatic nerve (E–H). Symbols of decreasing graytone intensities denote type C, B, and A neurons, respectively.

TRPV1 protein with Western blotting of the L5 DRGs relating to the capsaicin-treated sciatic nerves. The TRPV1 protein was markedly decreased already 3 days after the capsaicin treatment and remained at that low level amounting about 30% of the control throughout the entire period of the study. The long-lasting, apparently irreversible functional impairments observed after perineural capsaicin treatment, such as the abolition of chemogenic pain and neurogenic inflammation, elevated latencies of thermal nociceptive reflexes, and reduced thermal hyperalgesia, are in accord with the down-regulation of TRPV1 protein in the DRG neurons.

Several factors must be considered in the interpretation of the disparate changes brought about by the two types of nerve injuries, which differ substantially in their nature, that is, nerve transection and perineural capsaicin treatment. Nerve transection, classified as neurotmesis (Seddon, 1943), results in complete severance of the nerve. In contrast, although leading to a selective chemodenervation of nociceptive afferents which express the TRPV1 by a mechanism which involves a slowly progressing dying-back type of degeneration process (Jancsó and Lawson, 1990; Jancsó, 1992), perineural treatment with capsaicin leaves the nerve fibers continuous. The exact nature of this denervation process is still unclear, but it has been demonstrated that, although practically all capsaicin-sensitive C-fiber afferents are functionally inactivated, only about half of this population undergo degeneration, the number of unmyelinated axons in capsaicin-treated nerves decreasing by only some 30% (Jancsó and Lawson, 1990; Pini et al., 1990; Jancsó, 1992). This may imply that after perineural capsaicin, unlike after nerve transection, the surviving axons may provide some trophic support for the chemically injured neurons, which may be sufficient to promote the transcription, but not the translation of TRPV1 mRNA. This assumption is supported by find-

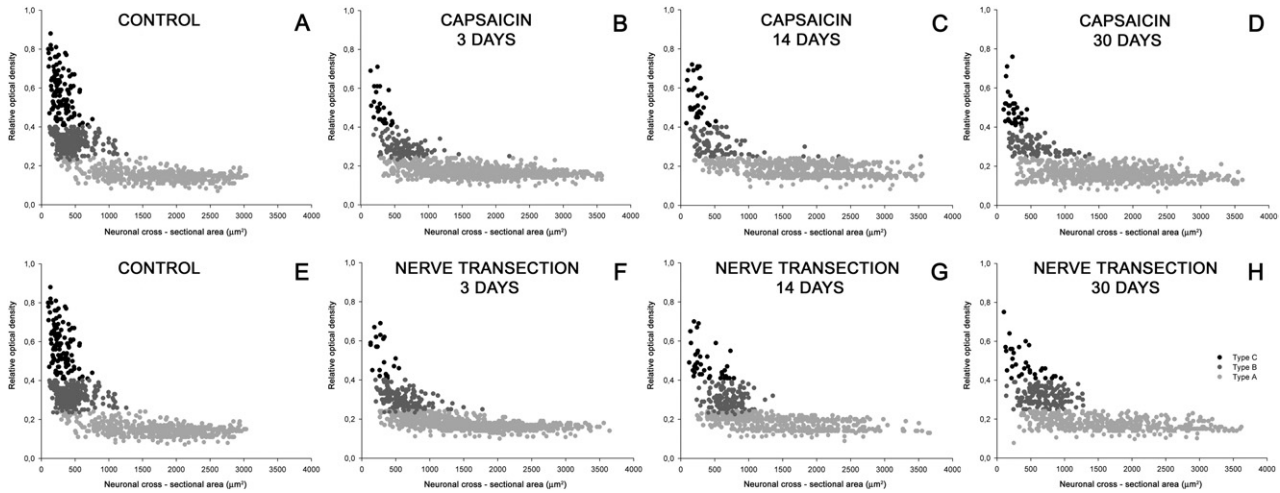


Fig. 5. Scatter plots showing the time course of changes in the populations of TRPV1-immunoreactive L5 DRG neurons following perineural capsaicin treatment (A–D) and transection of the ipsilateral sciatic nerve (E–H). Symbols of decreasing graytone intensities denote type C, B, and A neurons, respectively.

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

ings indicating that perineural capsaicin treatment exerts a profound selective, but transient blockade of axonal transport processes in C-fiber primary afferent neurons (Gamse et al., 1982; Sántha and Jancsó, 2003). Nerve growth factor (NGF) reaching the perikarya of the DRG neurons through retrograde axonal transport has been shown to play a pivotal role in the regulation of the expression of TRPV1 mRNA and protein in DRG neurons. Indeed, deprivation of DRG neurons of NGF under either *in vivo* or *in vitro* conditions has been shown to lead to a downregulation of TRPV1 mRNA expression and a loss of sensitivity to capsaicin (Winter et al., 1988; Aguayo and White, 1992; Jancsó and Ambrus, 1994; Jancsó et al., 1997; Michael and Priestley, 1999).

Similar phenomena involving a mismatch of mRNA and protein expressions have been reported, depending on the developmental and/or functional state of the DRG neurons. Peripherin mRNA and protein have been shown to be expressed in parallel in developing DRG neurons. However, in mature DRGs, large neurons express peripherin mRNA, but not the protein. This was attributed to changes in the availability of peripherally derived trophic factors such as NGF (Goldstein et al., 1996).

Although the distinct changes in the availability of trophic factors probably best explain, at least in part, the findings of the present study, other mechanisms may also be considered. The replacement of chemically injured neurons by proliferating DRG cells may offer an alternative possibility for the partial restitution of the neuron populations which express TRPV1 mRNA. Indeed, recent findings demonstrated a restoration of viscerosensory innervation by neurogenesis following a systemic injection of capsaicin (Czaja et al., 2008), which results in the degeneration of large populations of nodose and DRG neurons (Jancsó et al., 1977, 1980, 1985; Ritter and Dinh, 1988; Jancsó and Lawson, 1990; Jancsó, 1992; Hiura et al., 2002). However, this possibility seems unlikely, since little if any functional recovery was demonstrated after perineural treatment with capsaicin (Jancsó et al., 1980, 2011; Fitzgerald and Woolf, 1982; Jancsó and Lawson, 1990; Jancsó, 1992; Dux et al., 1999; Sántha and Jancsó, 2003).

The present study suggests that the regulation of the expression of TRPV1 after nerve injury is dependent on the type of the injury and not on the type of the DRG neuron. Whereas nerve transection resulted in an apparently long-lasting downregulation of TRPV1 mRNA expression, the selective chemodenervation of capsaicin-sensitive DRG neurons produced a transient and largely reversible downregulation of TRPV1 mRNA expression as shown by both *in situ* hybridization and quantitative RT-PCR. However, both treatments induced a seemingly irreversible inhibition of TRPV1 translation and/or changes in post-translational processing, resulting in a massive and permanent loss of TRPV1 protein from DRG neurons, as assessed by immunohistochemistry and Western blotting following perineural capsaicin treatment or nerve transection. The present findings may have important implications as concerns the mechanism(s) of chemically induced selective analgesia. The results point to the possibility that interfering with the translation and/or post-translational processing of nociceptive ion channels, such as the TRPV1, by using specific siRNAs, for example, may offer a novel approach to the production of antinociception by employing molecular biological tools.

Acknowledgments—This work was supported in part by OTKA PD 73259, TAMOP 4.2.1/B-09/1/KONV-2010-0005, and TAMOP 4.2.2/B-10/1-2010-0012. The authors are grateful to Z. Ambrus and É. Hegyeshalmi for their expert technical assistance and D. Durham for linguistic correction of the article.

REFERENCES

Aguayo LG, White G (1992) Effects of nerve growth factor on TTX- and capsaicin-sensitivity in adult rat sensory neurons. Brain Res 570:61–67.

Aldskogius H, Wiesenfeld-Hallin Z, Kristensson K (1988) Selective neuronal destruction by ricinus-communis agglutinin-I and its use for the quantitative-determination of sciatic-nerve dorsal-root ganglion-cell numbers. Brain Res 461:215–220.

Armitage P (2001) Biostatistics in clinical trial. Chichester: John Whiley.

Armitage P, Colton T (2005) Encyclopedia of biostatistics. Chichester: John Wiley.

Baranowski R, Lynn B, Pini A (1986) The effects of locally applied capsaicin on conduction in cutaneous nerves in four mammalian species. Br J Pharmacol 89:267–276.

Caterina MJ, Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 24:487–517.

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 288:306–313.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389:816–824.

Chung JM, Lee KH, Hori Y, Willis WD (1985) Effects of capsaicin applied to a peripheral nerve on the responses of primate spinothalamic tract cells. Brain Res 329:27–38.

Chung JM, Paik KS, Kim JS, Nam SC, Kim KJ, Oh UT, Hasegawa T, Chung K, Willis WD (1993) Chronic effects of topical application of capsaicin to the sciatic nerve on responses of primate spinothalamic neurons. Pain 53:311–321.

Czaja K, Burns GA, Ritter RC (2008) Capsaicin-induced neuronal death and proliferation of the primary sensory neurons located in the nodose ganglia of adult rats. Neuroscience 154:621–630.

Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 405:183–187.

Domoki F, Sántha P, Bari F, Jancsó G (2003) Perineural capsaicin treatment attenuates reactive hyperaemia in the rat skin. Neurosci Lett 341:127–130.

Donaldson LF, McQueen DS, Seckl JR (1995) Neuropeptide gene expression and capsaicin-sensitive primary afferents: maintenance and spread of adjuvant arthritis in the rat. J Physiol 486: 473–482.

Dux M, Sann H, Schemann M, Jancsó G (1999) Changes in fibre populations of the rat hairy skin after selective chemodenervation by capsaicin. Cell Tissue Res 296:471–477.

Fitzgerald M, Woolf CJ (1982) The time course and specificity of the changes in the behavioural and dorsal horn cell responses to noxious stimuli following peripheral nerve capsaicin treatment in the rat. Neuroscience 7:2051–2056.

Gamse R, Petsche U, Lembeck F, Jancsó G (1982) Capsaicin applied to peripheral nerve inhibits axoplasmic transport of substance P and somatostatin. Brain Res 239:447–462.

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058

Gibson SJ, McGregor G, Bloom SR, Polak JM, Wall PD (1982) Local application of capsaicin to one sciatic nerve of the adult rat induces a marked depletion in the peptide content of the lumbar dorsal horn. Neuroscience 7:3153–3162.

Goldstein ME, Grant P, House SB, Henken DB, Gainer H (1996) Developmental regulation of two distinct neuronal phenotypes in rat dorsal root ganglia. Neuroscience 71:243–258.

Green EC (1968) Anatomy of the rat. New York: Hafner.

Hiura A, Nakae Y, Nakagawa H (2002) Cell death of primary afferent nerve cells in neonatal mice treated with capsaicin. Anat Sci Int 77:47–50.

Holzer P (1991) Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 43:143–201.

Holzer P (2008) The pharmacological challenge to tame the transient receptor potential vanilloid-1 (TRPV1) nocisensor. Br J Pharmacol 155:1145–1162.

Jancsó G (1992) Pathobiological reactions of C-fibre primary sensory neurones to peripheral nerve injury. Exp Physiol 77:405–431.

Jancsó G (2009) Neurogenic inflammation in health and disease. In: Neuroimmune biology, Vol. 8 (Bérczi I, Szentiványi A, eds)Amsterdam: Elsevier.

Jancsó G, Ambrus A (1994) Capsaicin sensitivity of primary sensory neurones and its regulation. In: Peripheral neurons in nociception: physio-pharmacological aspects, Vol. 1 (Besson JM et al., eds), pp 71–87. Paris: John Libbey Eurotext.

Jancsó G, Dux M, Oszlács O, Sántha P (2008) Activation of the transient receptor potential vanilloid-1 (TRPV1) channel opens the gate for pain relief. Br J Pharmacol 155:1139–1141.

Jancsó G, Juhász A, Dux M, Sántha P, Domoki F (1997) Axotomy prevents capsaicin-induced sensory ganglion cell degeneration. Primary Sensory Neuron 2:159–165.

Jancsó G, Király E (1980) Distribution of chemosensitive primary sensory afferents in the central nervous system of the rat. J Comp Neurol 190:781–792.

Jancsó G, Király E, Jancsó-Gábor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270:741–743.

Jancsó G, Király E, Jancsó-Gábor A (1980) Direct evidence for an axonal site of action of capsaicin. Naunyn Schmiedebergs Arch Pharmacol 313:91–94.

Jancsó G, Király E, Joó F, Such G, Nagy A (1985) Selective degeneration by capsaicin of a subpopulation of primary sensory neurons in the adult rat. Neurosci Lett 59:209–214.

Jancsó G, Lawson SN (1987) Perineural capsaicin treatment of the sciatic-nerve in adult-rats causes transganglionic changes in the spinal-cord dorsal horn. J Physiol 394:109–109.

Jancsó G, Lawson SN (1988) Ganglionic changes associated with transganglionic degeneration of capsaicin-sensitive primary sensory afferents—a quantitative morphometric and immunohistochemical study. Reg Peptides 22:97–97.

Jancsó G, Lawson SN (1990) Transganglionic degeneration of capsaicin-sensitive C-fiber primary afferent terminals. Neuroscience 39:501–511.

Jancsó G, Oszlács O, Sántha P (2011) The capsaicin paradox: pain relief by an algesic agent. Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistry 10:52–56.

Jancsó G, Such G (1983) Effects of capsaicin applied perineurally to the vagus nerve on cardiovascular and respiratory functions in the cat. J Physiol 341:359–370.

Jancsó G, Such G, Rödel C (1987) A new approach to selective regional analgesia. In: Trends in cluster headache (Sicuteri F, Vecchiet L, Fanciullacci M, eds), pp 59–68. Amsterdam, New York: Excerpta Medica.

Jancsó N (1968) Desensitization with capsaicin as a tool for studying the function of pain receptors. In: Pharmacology of pain (Lim RKS, ed), pp 33–55. Oxford: Pergamon Press.

Julius D, Basbaum AI (2001) Molecular mechanisms of nociception. Nature 413:203–210.

Kasama S, Kawakubo M, Suzuki T, Nishizawa T, Ishida A, Nakayama J (2007) RNA interference-mediated knock-down of transient receptor potential vanilloid 1 prevents forepaw inflammatory hyperalgesia in rat. Eur J Neurosci 25:2956–2963.

Kissin I, Bright CA, Bradley EL Jr. (2002) Selective and long-lasting neural blockade with resiniferatoxin prevents inflammatory pain hypersensitivity. Anesth Analg 94:1253–1258.

Kissin I, Freitas CF, Mulhern HL, DeGirolami U (2007) Sciatic nerve block with resiniferatoxin: an electron microscopic study of unmyelinated fibers in the rat. Anesth Analg 105:825–831.

Knotkova H, Pappagallo M, Szállási A (2008) Capsaicin (TRPV1 agonist) therapy for pain relief: farewell or revival? Clin J Pain 24:142–154.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275.

Maggi CA, Meli A (1988) The sensory-efferent function of capsaicin-sensitive sensory neurons. Gen Pharmacol 19:1–43.

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. NY: Cold Spring Harbor Laboratory.

Michael GJ, Priestley JV (1999) Differential expression of the mRNA for the vanilloid receptor subtype 1 in cells of the adult rat dorsal root and nodose ganglia and its downregulation by axotomy. J Neurosci 19:1844–1854.

Nagy JI, Hunt SP (1983) The termination of primary afferents within the rat dorsal horn: evidence for rearrangement following capsaicin treatment. J Comp Neurol 218:145–158.

Oszlács O, Sántha P, Jancsó G (2009) Long-lasting antinociceptive and anti-inflammatory effects of *N*-oleoyldopamine, an endogenous vanilloid. Neuropeptides 43:413–413.

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45.

Pini A, Baranowski R, Lynn B (1990) Long-term reduction in the number of C-fibre nociceptors following capsaicin treatment of a cutaneous nerve in adult rats. Eur J Neurosci 2:89–97.

Pospisilova E, Palecek J (2006) Post-operative pain behavior in rats is reduced after single high-concentration capsaicin application. Pain 125:233–243.

Ritter S, Dinh TT (1988) Capsaicin-induced neuronal degeneration: silver impregnation of cell bodies, axons, and terminals in the central nervous system of the adult rat. J Comp Neurol 271:79–90.

Sántha P, Jancsó G (2003) Transganglionic transport of choleragenoid by capsaicin-sensitive C-fibre afferents to the substantia gelatinosa of the spinal dorsal horn after peripheral nerve section. Neuroscience 116:621–627.

Seddon HJ (1943) Three types of nerve injury. Brain 66:237–246.

Tohda C, Sasaki M, Konemura T, Sasamura T, Itoh M, Kuraishi Y (2001) Axonal transport of VR1 capsaicin receptor mRNA in primary afferents and its participation in inflammation-induced increase in capsaicin sensitivity. J Neurochem 76:1628–1635.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21:531–543.

Welk E, Petsche U, Fleischer E, Handwerker HO (1983) Altered excitability of afferent C-fibres of the rat distal to a nerve site exposed to capsaicin. Neurosci Lett 38:245–250.

Winter J, Forbes CA, Sternberg J, Lindsay RM (1988) Nerve growth factor (NGF) regulates adult rat cultured dorsal root ganglion neuron responses to the excitotoxin capsaicin. Neuron 1:973–981.

Yip HK, Rich KM, Lampe PA, Johnson EM (1984) The effects of nerve growth-factor and its antiserum on the postnatal-development and survival after injury of sensory neurons in rat dorsal-root ganglia. J Neurosci 4:2986–2992.

(Accepted 29 October 2011)

Please cite this article in press as: Szigeti C, et al., Disparate changes in the expression of transient receptor potential vanilloid type 1 receptor mRNA and protein in dorsal root ganglion neurons following local capsaicin treatment of the sciatic nerve in the rat, Neuroscience (2011), doi: 10.1016/j.neuroscience.2011.10.058